THE CYTOLOGY OF POLLEN ABORTION IN T (TEXAS) CYTOPLASMIC MALE-STERILE CORN (*Zea mays* L.)

BY
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A cytological comparison of anther development, with both light and electron microscopes, was made with T (Texas) cytoplasmic male-sterile, maintainer, and restored versions of inbred corn (*Zea mays* L.). Under the light microscope, the first abnormalities in sterile anthers appear at about the same time microspores are released from the tetrad. The tapetum thickens, stain less intensely, and small vacuoles appear. During later anther development, the vacuoles increase in size, and the tapetal cells become irregular and may rupture. Other abnormalities, such as differences in exine and intine development, microspore vacuolation, nuclear division, starch deposition, endothecial thickening, vascular differences, and anther dehiscence, appear at later stages and are thought to be secondary effects.

Studies of organelles with the electron microscope revealed mitochondrial abnormalities in the tapetum and

middle layers of sterile anthers between meiosis and tetrad stages. Mitochondria in these tissues lose their cristae, become disorganized internally, and swell. This does not occur in fertile anthers. Other organelles, including proplastids, endoplasmic reticulum, and ribosomes do not differ in fertile and sterile anthers until general degenerative changes begin. It is believed this breakdown of mitochondria is directly related to the tapetal abnormalities noted under the light microscope.

Measurements of size and counts of mitochondria and proplastids in fertile and sterile anther tissues revealed no differences that could be related in a causal way to sterility. A rapid division of mitochondria was observed, however, in the tapetum and sporogenous cells during early growth stages. This division did not occur in other anther cells, nor was it observed in proplastids. It is speculated that N (normal) and T (Texas) mitochondria may respond differently to the stress of division in the anther, and this may lead to pollen abortion.

Mitochondrial breakdown occurred in F_6T and $F_{44}T$ anthers and thus is not unique to either of these genomes. Mitochondria in the tapetum of restored $F_{44}T$ plants are normal. Breakdown thus is not inherent in the T cytoplasm, but is controlled by cytoplasm-nuclear interaction. No differences were observed in the mitochondria of leaves or nucellar cells of fertile and male-sterile plants.

Section 1

INTRODUCTION

Cytoplasmic male sterility is widespread among higher plants. Laser and Lersten (1972) list published reports of male sterility in some 140 species, including 47 genera and 20 families of angiosperms. Interest in this abnormality is high and arises both from scientific curiosity and from the practical value of male sterility in production of hybrid seed without emasculation.

In cytoplasmic male-sterile plants, pollen aborts in the anther, thus making them male sterile; however, female structures usually are not affected. This abnormality is conditioned by determinants in the cytoplasm, is maternally inherited, and has been termed "cytoplasmic male sterility" to distinguish it from "genetic male sterility," which is inherited in normal Mendelian fashion. Fertility may be restored by the introduction of nuclear genes, termed "restorers," through the pollen. Although some differences in vegetative characters of fertile and sterile plants have been observed, separation of fertile and sterile plants before flowering is difficult or impossible. Duvick

(1965), Edwardson (1970), Harvey et al. (1972), and Laser and Lersten (1972) have published excellent and comprehensive reviews on cytoplasmic pollen sterility.

With the exception of a preliminary report (Lee, 1973) the last comprehensive comparison of anther development in cytoplasmic male-sterile and fertile corn (*Zea mays* L.) was made more than 20 years ago (Chang, 1954), and that study relied solely on the light microscope. With the notable improvements in cytological techniques since that time, such as stained semithin plastic sections for the light microscope and ultrathin sections prepared for the electron microscope it seemed desirable to reinvestigate male sterility in corn. Also, the growth in importance and interest in male sterility in the production of hybrid seed and the demonstrated involvement of the T (Texas) male-sterile cytoplasm in the nationwide outbreak of southern corn leaf blight in 1970 have made a reinvestigation doubly important.

Anthers were chosen for study because the first observable differences between fertile and sterile plants (tapetal abnormalities) occur in these organs and because the chief deviation from normal development (pollen abortion) also occurs there. It is recognized, of course, that cytoplasmic male sterility is maternally inherited and thus must be transmitted from one generation to the next through the female.

Although a cytological approach can seldom reveal basic physiological mechanisms or clarify cause and effect relationships, it may well offer important clues to such questions. By use of improved procedures, it was hoped that an understanding of the degenerative processes in sterile anthers could be extended from the gross tissue level to the level of the cytoplasmic organelle. The present study was undertaken as an effort to attain this goal.

Section 2

LITERATURE REVIEW

2.1 Comparison of Fertile and Sterile Anthers

Numerous cytological comparisons of anther development in normal and cytoplasmic male-sterile plants have been made to determine the nature and time of pollen breakdown. These may be classified under the following headings:

2.1.1 Meiosis

Meiosis in microspore mother cells of sterile plants generally is normal; however some species were reported with irregular meiosis and/or breakdown of microspore mother cells. These include wheat (Chauhan and Singh, 1966), orchardgrass (Filion and Christie, 1966), barley (Kaul and Singh, 1966), carrot (Zenkteler, 1962), cotton (Murthi and Weaver, 1974), and nightshade (Grun and Aubertin, 1966).

2.1.2 Callose

Abnormal callose behavior with cohesion of microsporocytes was reported in petunia (Frankel et al., 1969), sorghum (Damon, 1961; Warmke and Overman, 1972).

In sweet pepper (Horner and Rogers, 1974), the tetrad of microspores collapses while still encased in callose.

2.1.3 Tapetum

Tapetal abnormalities in sterile anthers have been reported in many species. These may be grouped under the following types:

i) Premeiotic tapetal degeneration. Species belonging to this type are: wheat (Chauhan and Singh, 1966), sweet pepper (Novak, 1971), and barley (Kaul and Singh, 1966).

ii) Postmeiotic tapetal degeneration. This has been found to occur in ryegrass (Nitzche, 1971), orchardgrass (Filion and Christie, 1966), sorghum (Overman and Warmke, 1972), corn (Lee, 1973), sweet pepper (Horner and Rogers, 1974), and sugar beet (Artschwager, 1947).

iii) Persistant tapetum. A number of species have been reported as belonging to this group: corn (Chang, 1954), wheat (Chauhan and Singh, 1966; Joppa et al., 1966), sudangrass (Alam and Sandal, 1967), sorghum (Brooks et al., 1966; Narkhede et al., 1968; Singh and Hadley, 1961; Webster and Singh, 1964), flax (Dubey and Singh, 1965), barley (Kaul and Singh, 1966), sunflower (Nakashima and Hosokawa, 1974), ryegrass (Hayward and Manthriratna, 1972), shallot (Singh and Kobabe, 1969), pearl millet (Reddy and Reddi, 1974), and sugar beet (Artschwager, 1947).

At the electron microscope level, Overman and Warmke (1972) found that the primary tapetal inner wall in sorghum does not disappear in sterile anthers, and a secondary wall is deposited. They suggested this possibly presents a barrier to nutrient transfer to the locule.

2.1.4 Endothecium

Differences between fertile and sterile plants also have been noted in other anther tissues. In male-sterile orchardgrass (Filion and Christie, 1966), the endothecium enlarges but does not develop fibrous bands. No endothecial thickening was found in the following cytoplasmic male-sterile lines: wheat (Chauhan and Singh, 1966), carrot (Zenkteler, 1962), orchardgrass (Filion and Christie, 1966), barley (Kaul and Singh, 1966), geranium (Harney and Kung, 1967), and bean (Pritchard and Hutton, 1972). Reddy and Reddi (1974) reported that a thicker endothecium is associated with male sterility in pearl millet.

2.1.5 Vascular tissue

Abnormalities in anther vascular structure were reported in wheat (Rao and Jain, 1975; Joppa et al., 1966), and sugar beet (Rohrbach, 1965). Laser (1972 a,b) did not observe differences in vascular structure in fertile and sterile sorghum anthers.

2.1.6 Organelles

i) Mitochondria. Differences between fertile and sterile plants at the subcellular level were first described in corn by Rhoades (1933). He observed differences in cytoplasmic elements of microspores. De Vries and Ie (1970) observed fewer organelles in sterile pollen grains of wheat. These were less active and thus led to degeneration.

Palilova et al. (1967) reported differences in structure of mitochondria in nucellar cells in cytoplasmic male-sterile and fertile lines of corn. Normal mitochondria, however, were found in restored lines (Turbin et al., 1968).

Levings and Pring (1976) treated mitochondrial DNA from corn with restriction endonuclease and separated the fragments by electrophoresis. They found consistent differences in the position of bands from fertile and sterile seedlings.

Mitochondria also have been related to male sterility by a number of recent studies on the reaction of mitochondria from fertile and sterile plants to the toxin produced by Bipolaris (Helminthosporium) maydis, race T. Mitochondria from fertile plants are not affected by the toxin; those from sterile plants (which also are highly susceptible to southern corn leaf blight) swell irreversibly (Miller and Koeppe, 1971; Flavell, 1975; Barratt and Flavell, 1975).

ii) Plastids. Working with interspecific *Epilobium* hybrids, Michaelis (1969) has shown that plastids of the Essen strain contain factors that interact with *parviflorum* genes to induce male sterility. Chen et al. (1975) extracted fraction I protein from the chloroplasts of fertile and male-sterile *Nicotiana* plants. Polypeptides from this protein formed distinctive patterns after electrophoresis and strongly suggest that chloroplasts are involved in male sterility in *Nicotiana*.

2.1.7 Viruses

Atanasoff (1971, 1974) suggested that cytoplasmic male sterility should be attributed to viral infections. No convincing experimental evidence for viruses in sterile plants has been presented, however. Graft transmission of male sterility in petunia (Frankel, 1956, 1962; Edwardson and Corbett, 1961; Bianchi, 1963), and in sugar beet (Curtis, 1967), however, is suggestive of viral involvement. Recently, Edwardson et al. (1976) have found unidentified cytoplasmic spherical bodies (70 nm in diameter) in male-sterile broad bean but not in maintainer, restorer, or restored lines.

2.2 Cytology of Normal Anthers

In order to compare abnormal anther development in sterile plants with normal anther development, an understanding of normal development is essential. This is demonstrated by the effort workers investigating male sterility have invested in establishing normal developmental patterns for the species under study: in sugar beet (Hoefert, 1969, 1971), sorghum (Overman, 1969; Christensen et al., 1972), and lemon (Horner and Lersten, 1971).

Fortunately, we have a rich and growing literature on microsporogenesis in normal, fertile angiosperms. Much of this is recent and involves use of the electron microscope. Several excellent reviews also are available (Maheshwari, 1949; Vasil, 1967; Heslop-Harrison, 1972; Mascarenhas, 1975).

Specific areas of normal anther development have been explored in a number of recent studies. The most important of these are listed below:

2.2.1 Normal Callose Development

Studies on callose development in fertile anthers have been reported in various species (Gorska-Brylass, 1967, 1970), Zea (Skvarla and Larson, 1966), Helleborus (Echlin and Godwin, 1968 b), Sorghum (Horner and Lersten, 1970; Overman, 1969), Lilium (Heslop-Harrison, 1966;

Heslop-Harrison and Mackenzie, 1967), Tradescantia (Mepham and Lane, 1969a), Cannabis (Heslop-Harrison, 1964), and Desmodium (Buss et al., 1969).

2.2.2 Normal Tapetal Development

Studies on tapetal development in fertile anthers have been reported in Lilium (Heslop-Harrison and Dickinson, 1969), grasses (Banerjee, 1967), Sorghum (Christensen and Horner, 1970; Horner and Lersten, 1971), Helleborus (Echlin and Godwin, 1968a), Poa (Rowley, 1963), Allium (Risueno et al., 1969), Podocarpus (Vasil and Aldrich, 1970), Tradescantia (Mepham and Lane, 1969a,b), and Beta (Hoefert, 1971; Artschwager, 1947).

2.2.3 Normal Pollen Wall and Germ Pore Development

Studies of pollen wall development in fertile anthers have been reported in Helleborus (Echlin and Godwin, 1968b, 1969), Zea (Skvarla and Larson, 1966), Silene (Heslop-Harrison, 1963), Lilium (Heslop-Harrison, 1964, 1968; Dickinson and Heslop-Harrison, 1968, 1971), Sorghum (Horner and Lersten, 1970, 1971; Christensen et al., 1972; Christensen and Horner, 1974), Tradscantia (Mepham, 1970; Mepham and Lane, 1968), Passiflora (Larson, 1966), Tilia (Chambers and Godwin, 1961), Parkinsonia (Larson and Lewis, 1961); (Heslop-Harrison, 1971), Podocarpus (Vasil and Aldrich, 1970), and Cannabis (Heslop-Harrison, 1964).

2.2.4 Normal Organelle Development

Normal organelle development and behavior have been presented in the following excellent reviews:
Kirk and Tilney-Bassett (1967), Opik (1968), Reinert and Ursprung (1971), Malone et al. (1974), Munn (1974), and Birky et al. (1975).

Section 3

MATERIALS AND METHODS

3.1 Plant Materials

The corn lines used include F_{44} and F_6 maintainer lines and their T cytoplasmic male-sterile counterparts. These lines were highly inbred, the maintainer lines having been selfed and crossed repeatedly to male-sterile plants. The stocks thus were essentially isogenic for the F_6 or F_{44} nuclear genomes and differed only in having N (normal) or T (Texas) male-sterile cytoplasms and in bearing fertile pollen (N plants) or completely aborted pollen (T plants). Hybrids between $F_{44}T$ and K_{55} (restorer) also were used. In the accompanying figures and tables, materials from F_{44} maintainer and cytoplasmic male-sterile plants are termed "fertile" and "sterile," respectively. When other lines are used, special indication is made.

3.2 Tissue Preparation

The following organs were prepared for study:

3.2.1 Anthers

Tassels of different ages were collected from the field at Gainesville in June 1974 and 1975. Individual spikelets were carefully removed and separated under a dissecting microscope. Each spikelet contains two functional flowers, one of which is generally more advanced than the other. The anthers were removed, placed on a piece of filter paper soaked with fixative (Karnovsky, 1965, plus a trace of Aerosol OT) and cut crosswise with a razor blade to facilitate penetration. Cut anthers were subsequently transferred to vials of fresh fixative, fixed for 6 hours at room temperature, rinsed in 0.05M cacodylate buffer at pH 7.2 and postfixed overnight in 2 percent OsO_4 in cacodylate buffer. Anthers were dehydrated in an ethanol series, passed through acetone, and flat embedded in Spurr plastic (Spurr, 1969). Selected anthers were cut from blocks and mounted on specimen slugs (Pelco) with epoxy glue.

3.2.2 Leaf Tissues

Leaves of various ages from both fertile and sterile plants were cut crosswise in strips about 1 mm in width. These were subdivided into segments 3-4 mm in length, and prepared for sectioning by the same procedures employed for anthers.

3.2.3 Ovules

Young ear shoots were collected from plants grown in the greenhouse. Husks and silks were removed, and rows of young female flowers were dissected by making longitudinal cuts with a sharp razor blade. Young kernels were separated and trimmed as closely as possible to aid fixative penetration. Fixation and embedding procedures were similar to those used for anthers.

3.3 Procedures For Light Microscopy

Plastic-embedded tissues were mounted and sectioned at a thickness of 1-1.5 μm, using a diamond knife and Porter-Blum MT-1 ultramicrotome. Staining was performed by a procedure developed during the course of the present study but published elsewhere (Warmke and Lee, 1976).

These stained semithin sections of fertile and sterile anthers were studied under the light microscope and were arranged in two closely paired series of developmental stages: precallose, central callose, meiosis, tetrad, young microspore, and intermediate microspore. These probably correspond quite closely with stages C_2, C_3, C_4, P_1, P_2 and P_3 reported by Moss and Heslop-Harrison (1967). Later stages that could not be paired because of microspore abortion were late microspore stage and collapsed locule stage in sterile anthers and vacuolate 1-nucleate microspore, 2-nucleate young pollen, and mature pollen in fertile anthers.

Photomicrographs were made with a Bausch and Lomb 4x5 integrated camera, mounted on a Bausch and Lomb Dynazoom Photobinocular microscope. Polaroid positive-negative type 55 film was used.

3.4 Electron Microscopy

The materials fixed, embedded, and mounted for semithin sections also were used for electron microscopy. Ultrathin sections were cut at a thickness of 60 to 100 nm with a diamond knife and a Porter-Blum MT-1 ultramicrotome and placed on copper grids coated with Formvar and carbon. A triple stain procedure (Soloff, 1973) was used to increase electron density. The steps included: 0.9 percent $KMnO_4$ in 0.2M sodium phosphate buffer at pH 6.5, 1 percent uranyl acetate in 50 percent ethanol (made from absolute ethanol) and lead citrate (Reynolds, 1963). Staining time depends on the material, but 2-5 minutes for each step has been found generally satisfactory. Thorough rinsing with distilled water between steps was found to be necessary to avoid stain precipitation.

The sections were studied and photographed with a Philips EM 200 electron microscope, using Eastman Kodalith 35mm film.

3.5 Morphometric Analysis

3.5.1 Tissues Studied

An estimate of the size and number of mitochondria and proplastids per unit volume in the anther tissues studied at the various stages of anther development was obtained. Organelle number per cell also was calculated for tapetum and sporogenous tissues.

3.5.2 Methods Employed

Tissues were micrographed on 35 mm film at an initial magnification of approximately 3900 X and then projected on a white screen at a final magnification of 100,000 X. Ten centimeters on the screen thus represented 1 μm of actual tissue distance. A diffraction grating with spacing of 0.000463 mm was used to correct for minor variation in initial magnification. The number and two dimensions (major and minor axes) of mitochondria and proplastids occupying a 50 cm^2 area on the projection screen for each anther tissue, type, and age were measured and recorded.

Two replications (anthers) and two or three subsamples (areas) were used for each tissue at each developmental stage for fertile and sterile plants. The number of organelles per subsample thus could vary from 0 or 1 to more than 30.

i) Estimation of organelle volume. The volume of mitochondria and plastids was determined by using the formula for the volume of an ellipsoid (prolate): volume = $\frac{4}{3}\pi ab^2$, where a = the major semiaxis and b = the minor semiaxis.

ii) Estimation of organelle number per unit volume. The number of mitochondria or plastids per unit volume was obtained by applying the equation of Weibel and Gomez (1962) and Weibel (1963)

$$N_V = \frac{K}{\beta} \frac{N_A^{3/2}}{V_V^{1/2}}$$

K is the distribution coefficient of particles. In general, the value of K lies most often within the range of 1.01-1.10, and an arbitrary estimate of K=1.05 can be made for practical purposes (Weibel, 1963). The coefficient β varies according to the axial ratio of the particles. The plot of β as a function of axial ratio $\lambda = \frac{L}{D}$ for spheroids and cylinders has been deduced by Weibel (1969) (relation of β and λ listed in Apendix I). N_A is defined as the number of mitochondria or plastids per unit area of cytoplasmic profile. The parameter V_V is the volume proportion of cytoplasm occupied by the organelles. Instead of considering the thickness of cytoplasm to obtain the volume fraction of these organelles, V_V can be determined as A_A, the areal fraction of particles. This principle ($A_A = V_V$) was developed by the French geologist, Delesse (1847) and is now widely accepted.

iii) Estimation of cell volume. Cell volume in anther tissues was calculated by use of semithin sections, the light microscope, and an ocular micrometer. Total sectional area of sporogenous tissue was obtained by averaging two diameters in locule cross sections and calculating the area from this. The area of the ring of tapetal cells was calculated by subtracting the area of sporogenous tissue from the area included within the outside tapetal circumference. Cell area in both tissues was calculated by dividing total tissue area by number of cells included. Cell length was obtained by measuring length of cells in longitudinal sections of anthers of corresponding ages. Finally, cell volume was calculated as the product of cross-sectional area and cell length at each developmental stage.

iv) Estimation of organelle number per cell. These calculations were obtained by multiplying the average number of organelles per unit volume obtained in (ii), by mean cell volume, obtained in (iii). Where cell division had taken place, a cell division factor was calculated from the ratio of cell number in tissues at each stage compared to cell number in the youngest stage.

3.5.3 Computer Programs

Due to the large amount of data involved and the complicated formula for estimating organelle number per unit volume, data reducing was done by computer.[1] A Fortran program was written to compute organelle volume and number per unit volume (see Appendix II for programs).

3.5.4 Statistical Analysis

Analysis of variance was used in analysis of organelle volume for fertile vs. sterile lines and for the various stages of anther development in each tissue. A split plot design was used in the analysis of combined cytoplasms, stages, and tissues. Because cross sectional areas and cell lengths could not be taken from the same cell, estimates of statistical significance for factors requiring cell volume (which involves products of lengths and areas) could not be made. The SAS computer program (Statistical Analysis System, Service et al., 1972) was used for the anaysis (see Appendix III for programs). Duncan's multiple range test at the 5 percent level of probability was used to detect significant differences among means.

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[1] Northeast Regional Data Center of the State University System of Florida.

Section 4

RESULTS AND DISCUSSION

4.1 Light Microscope Comparison of Anther Development In Fertile and Male-Sterile Corn Lines

Semithin sections (1.0-1.5 μm in thickness) were cut from plastic-embedded anthers and stained for study under the light microscope (Warmke and Lee, 1976). These served as an orientation to the overall process of anther development and made possible the selection of fertile and sterile anthers for comparison at the electron microscope level. This work closely parallels a previous study made by use of paraffin sections (Lee, 1973).

Photomicrographs of anther sections at various developmental stages are shown in Fig. 1-9. The left half of each figure (a) is from a fertile anther, and the right half (b) is from a sterile anther at approximately the same stage of development. Fig. 1-6 are the same stages examined ultrastructurally in Sections 4.2 and 4.3.

4.1.1 Precallose (S_1)

Figures 1a,b show young fertile and sterile anthers. The four wall layers (epidermis, endothecium, middle layer, and tapetum) have been formed, as well as a central column of sporogenous cells. At this stage, mitotic divisions of sporogenous cells have stopped, but callose formation has not yet begun. These cells are large, have large nuclei and dark nucleoli, and at this stage may be termed microspore mother cells (MMC).

4.1.2 Central Callose (S_2)

Figures 2a,b show halves of fertile and sterile anthers after callose formation has begun. Callose is deposited between the plasma membranes and cell walls, first along the inner margins of the MMC's (Fig. 2), and later so that it completely surrounds each cell. Callose deposition always is heavier, however, toward the center of the locule than at the outer boundaries.

Callose stains intense blue after the methylene blue combination and bright red after the hematoxylin-safranin procedure. There does not appear to be a difference in amount or staining reaction of callose in fertile and sterile anthers at this time. Subsequently, the heavy central callose splits along the original cell boundaries, and the sectors separate with the MMC's.

Shortly before the first meiotic metaphase, the tapetal cells undergo mitosis without cytokinesis and become binucleate. This division is more or less synchronous in all tapetal cells of a locule section and occurs in both fertile and sterile anthers.

4.1.3 Meiosis (S_3)

Figures 3a,b show fertile and sterile anthers at first meiotic metaphase. The meiotic divisions appear normal in both fertile and sterile anthers and result in the formation of dyads and then tetrads of cells. Cytoplasmic organelles (small dark spheres) and vacuoles (small clear areas) now are seen in the sporogenous cells in both fertile and sterile anthers.

The tapetum tends to stain darkly during the meiotic divisions and increases in thickness. The cells in the outer anther layers become vacuolate and stain lightly.

4.1.4 Tetrad (S_4)

The young microspores are separated by partition walls, which stain bright red with the procedures used. The callose walls stain very lightly at this stage and surround both the original tetrad and the individual microspores.

The tapetum continues to thicken and is filled with cytoplasm and organelles. The entire locule has increased in diameter (Fig. 4a,b), and intercellular space appears at both the center of the locule and at the margins.

Up to this time, sectioned fertile and sterile anthers are very similar in size and structure and probably cannot be distinguished under the light microscope.

4.1.5 Young Microspore (S_5)

As the result of callose breakdown, the young microspores are liberated into the locule cavity (Fig. 5a,b). They tend to be somewhat irregular in outline when first released, and the cytoplasm is filled with small vacuoles. The young microspores in fertile and sterile anthers appear similar at this stage.

The tapetal cells in fertile and sterile anthers at this time, however, are different. In sterile anthers (Fig. 5b) the tapetum is thicker, stains somewhat less intensely, and has developed small vacuoles.

4.1.6 Intermediate Microspore (S_6)

Here the differences noted in the preceding stage are accentuated: the tapetum has become more vacuolate and irregular and stains lightly (Fig. 6b). In fertile anthers the tapetal cells are regular, columnar in shape, have few vacuoles, and stain darkly (Fig. 6a). The walls of the tapetum toward the locular cavity are lined with Ubisch bodies which stain a conspicuous blue green after the methylene blue procedure. These form in both fertile and sterile anthers but appear somewhat more pronounced in the former.

At this time, differences also are noted in microspore development. In fertile anthers, they are regular in outline and have thick walls. In sterile anthers, the walls are thinner and the spore outlines more irregular. There is no difficulty in distinguishing sectioned fertile from sectioned sterile anthers under the light microscope at this time.

Portions of locules from fertile and sterile anthers at higher magnification are shown in Fig. 10a,b.

4.1.7 Late 1-Nucleate Microspore

Figures 7a,b show fertile and sterile anthers at a later stage of development. It is difficult to pair anthers of the same age at this and later stages, however, because of the great differences in developmental patterns. In fertile anthers, the microspores still have a single large nucleus, but have greatly enlarged, have thick exines, and one large central vacuole. The tapetum, although cellular and basophilic, is compressed into a scalloped peripheral band by the enlarging microspores.

Development appears to have stopped in the sterile anthers (Fig. 7b). Microspores are irregular in outline, have thin walls, and still contain many small vacuoles, instead of one large one as found in fertile anthers. Tapetal cells are vacuolate, irregular in shape, and some appear to have broken and discharged their contents into the locule cavity.

Sections of anthers at this stage are shown at a higher magnification in Fig. 11a,b.

4.1.8 Young Pollen

Microspore development continues in a normal manner in fertile anthers (Fig. 8a). The single large nucleus undergoes a mitotic division to form vegetative and generative nuclei. Cytoplasm is cut off around the generative nucleus, which then rounds up and becomes the small generative cell. The wall of this cell stains a light, iridescent red after safranin and is clearly seen in sections under the light microscope (Fig. 8a). The tapetum is further reduced and appears only as an occasional flattened cell.

Microspore nuclei in sterile anthers have not been observed to undergo division, and pairing fertile and sterile anthers at this stage thus is difficult. Sterile microspores never develop the single large vacuole; they remain thin walled and become very irregular in outline (Fig. 8b). Tapetal cells are irregular in shape, contain little cytoplasm, and frequently are broken.

4.1.9 Mature Pollen

As development continues, starch is deposited in fertile pollen (Fig. 9a). The central vacuole eventually disappears and is replaced by food reserves. Heavy wall layers are completed. These consist of a sculptured exine that stains a bright-blue green after the methylene blue procedure and a contrasting orange-red intine (Fig. 9a). Before anthesis, the generative cell divides to form a pair of elongate male gametes. A corn pollen grain at time of shedding thus has three cells: one large vegetative cell and two elongate sperm cells.

By maturity, the outer anther walls have undergone considerable change: the epidermis is highly cutinized, the endothecium develops special thickenings that aid in anther dehiscence, and the middle layer has disappeared or been compressed into a thin line. The tapetum also disappears in mature fertile anthers, leaving the tapetal wall and Ubisch bodies lining the locule cavity.

Finally, the septum between the two locules in each anther lobe breaks to form two large cavities in each anther. Anthesis occurs in the morning, the anthers are completely extruded and become pendant, and pollen is shed from slits that develop near the tip of each anther.

Sterile anthers are desiccated and collapsed at maturity; the septum between locules does not break.

All that remains of the microspores is partially developed exines that are folded and compressed into elongate masses (Fig. 9b), and the intine layer does not form. Under field conditions at Gainesville, endothecial thickening, anther exertion, and anther dehiscence do not occur.

4.1.10 Vascular Bundle

In corn, a single collateral vascular bundle runs through the filament and continues to near the anther apex. This bundle is not connected by any vascular elements to the anther locules, but is separated by parenchyma cells surrounding the vascular bundle and by ground parenchyma of the anther connective, across which all material to and from the vascular system must be transported (Fig. 12).

A number of previous workers have investigated vascular bundles of fertile and sterile anthers, in search of possible explanations of pollen abortion. Some have reported differences (Joppa et al., 1966; Rao and Jain, 1975) and others not (Laser, 1972a,b). In the present study, a slight difference in behavior of sheath cells surrounding the bundle has been noted, but not in the principal bundle elements.

Figures 13a,b show cross sections of vascular bundles of fertile and sterile anthers, respectively, at the tetrad stage. No differences are observed at this time, and xylem and phloem elements appear normal.

Figures 14a,b are corresponding photomicrographs at the intermediate microspore stage. The sheath cells surrounding the vascular bundle of the sterile anthers appear somewhat flattened and less turgid than those in fertile anthers.

Figures 15a,b show sections of vascular bundles of fertile and sterile anthers at late microspore stages. Here the differences are obvious, with the sheath cells open in the fertile, but collapsed in the sterile anthers.

Sheath cells of the fertile anthers remain turgid until pollen is shed; those in the sterile anthers collapse completely, along with other cells of the ground parenchyma. The vascular elements, themselves, appear normal at late stages in both fertile and sterile anthers.

4.1.11 Discussion

The improvements in procedures for cutting and staining plastic-embedded plant materials for the light microscope (see Warmke and Lee, 1976) have made possible a critical reevaluation of comparative microspore development in fertile and Tcms corn anthers. Not only can improved electron microscope fixatives be used, but thinner sections, which yield sharper images, are possible. In addition to the semithin sections cut for the light microscope, ultrathin sections may be cut from the same blocks for the electron microscope. This makes possible the processing of large numbers of anthers and selecting those at the same stage from fertile and sterile plants before going to the electron microscope. It also makes possible the detection and discarding of damaged tissues. These techniques have been used in very few previous studies of male sterility (Horner and Rogers, 1974; Christensen and Horner, 1970; Christensen et al., 1972; Skvarla and Larson, 1966). Other workers have employed thicker paraffin sections, and the soft embedment precludes cutting ultrathin sections from the same blocks.

Anthers are difficult cytological materials, however, even with the improved techniques. Uncut anthers, with their protective layer of cutin, are almost impervious to fixatives.

The first and probably most important difference between fertile and sterile anthers appears in the tapetum, at about the time microspores are released from the tetrad (Fig. 5b). In sterile anthers, the tapetum thickens, stains less intensely, and numerous small vacuoles appear. This process continues during the intermediate and late 1-nucleate microspore stages (Fig. 6b, 7b), when the vacuoles increase in size and the tapetal cells become irregular and may rupture. This is in sharp contrast to tapetal cell development in fertile anthers shown at the left in Fig. 1-9.

This course of events differs considerably from that reported in the last comprehensive comparison of fertile and male-sterile corn anthers (Chang, 1954). Chang found that tapetal cells in fertile anthers broke and discharged their contents into the locules and that tapeta in sterile locules persisted longer. He also reported significant differences in size of meiocytes and in tapetal thickness and cross-sectional area. No such differences between fertile and sterile anthers in size of sporogenous cells or in tapetal thickness or cross-sectional areas were found in the present study.

Collapse of sheath cells around the vascular bundles in sterile corn anthers, which begins at the intermediate microspore stage, has not been noted in fertile anthers. Tapetal abnormalities were first noted at the young microspore stage; this is slightly before collapse of sheath cells begins. Vascular abnormalities previously have been associated with male sterility by some workers (Joppa et al., 1966; Rao and Jain, 1975). No relation to sterility has been reported by others (Laser, 1972a,b).

The first abnormalities noted in Tcms corn anthers thus are in the tapetum: with enlargement, vacuolization, and lighter stain reaction. This is followed in sequence by other events, in the microspore and in the anther, which probably can be interpreted as secondary and resulting from arrested development. The evidence thus supports earlier investigations (see review of Laser and Lersten, 1972) and suggests that abnormalities leading to pollen abortion may originate in the tapetum in a number of male-sterile types.

Pollen abortion in T-cytoplasmic male-sterile corn is similar in many respects to that in male-sterile sorghum (Warmke and Overman, 1972; Overman and Warmke, 1972). It differs in that callose degenerates early in sterile sorghum, leaving the sporogenous cells unprotected by special callose walls. This leads to stickiness and fusion in sorghum. No early callose dissolution was noted in corn, and fusion of microspores is uncommon. Laser (1972b) observed no differences in the tapetum of fertile and sterile sorghum anthers until the time of microspore abortion.

4.2 Ultrastructural Comparison of Anther Development in Fertile and Male-Sterile Corn Lines

Descriptions of anther and microspore development in a number of monocot species have been given in several recent reports (Skvarla and Larson, 1966; Christensen et al., 1972; and De Vries and Ie, 1970). The present study, therefore, will be limited to those areas where fertile and

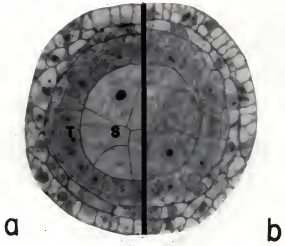


Figure 1. Stained semithin sections of fertile and sterile corn anthers at precallose stage. X 430. The left half (a) is from a fertile anther ($F_{44}N$); the right half (b) is from a sterile anther ($F_{44}T$). The anther consists of sporogenous cells (S), tapetum (T), middle layer, endothecium, and epidermis in that order.

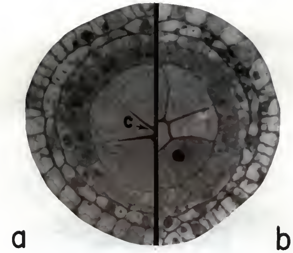


Figure 2. Semithin sections of fertile and sterile corn anthers at central callose stage. X 430. The left half (a) is from a fertile anther; the right half (b) from a sterile anther. (c) indicates callose.

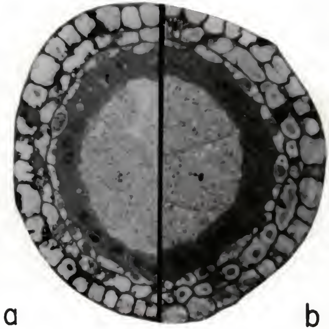


Figure 3. Sections of fertile and sterile corn anthers at meiosis. X 430. The left half (a) is from a fertile anther; the right half (b) from a sterile anther.

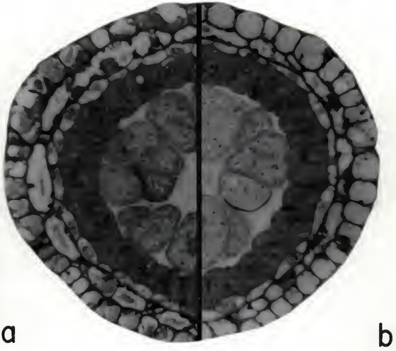


Figure 4. Sections of fertile and sterile corn anthers at tetrad stage. X 430. The left half (a) is from a fertile anther; the right half (b) from a sterile anther.

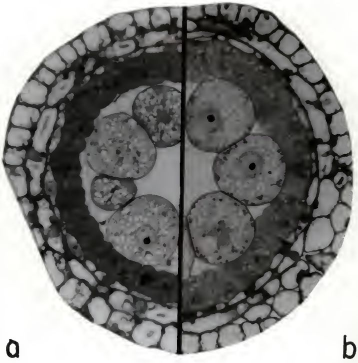


Figure 5. Sections of fertile and sterile corn anthers at young microspore stage. X 430. (a) and (b) are from fertile and sterile anthers, respectively. Note differences in thickness and stain reaction of fertile and sterile tapetal cells at this stage.

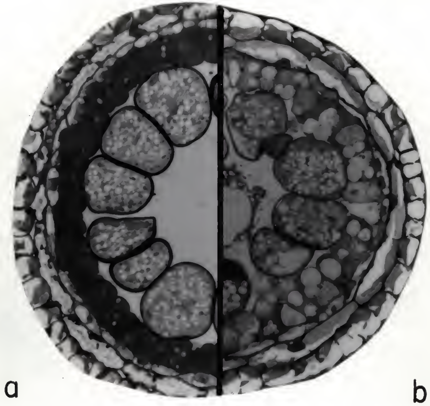


Figure 6. Sections of fertile and sterile corn anthers at intermediate microspore stage. X 430. (a) and (b) are fertile and sterile anthers, respectively. The tapetum of sterile anther becomes vacuolate and stains lightly.

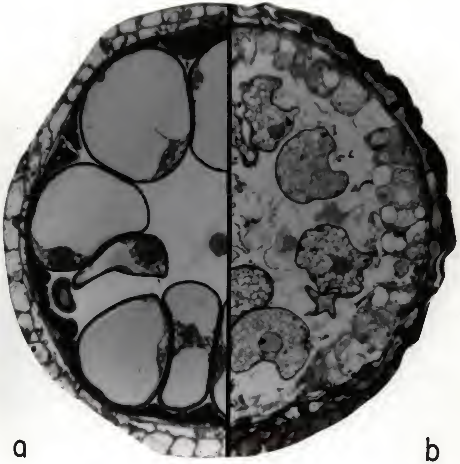


Figure 7. Sections of fertile and sterile corn anthers at late 1-nucleate microspore stage. X 430. Note tapetal disorganization and arrested microspore development in sterile half (b).

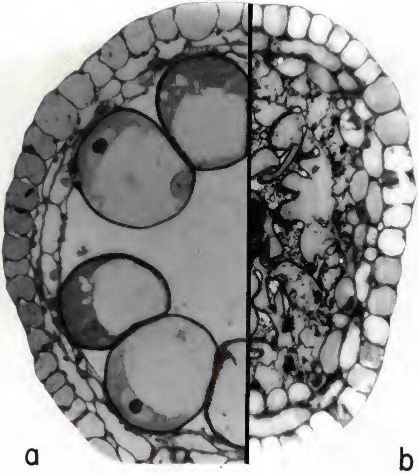


Figure 8. Sections of fertile and sterile corn anthers at young pollen stage. X 430. The sterile anther (b) is completely disorganized.

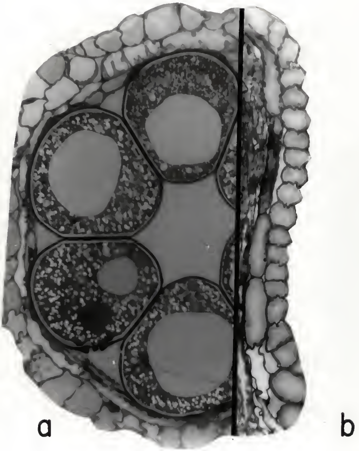


Figure 9. Sections of fertile and sterile corn anthers nearing maturity. X 430. Starch is being stored in pollen of fertile anther (a); sterile anther (b) has collapsed.

Figure 10. Sections of portions of fertile and sterile corn anthers at intermediate microspore stage at higher magnification. X 970. (a) is from fertile and (b) from sterile anther. Note vacuolation of sterile tapetum and adhesion of young microspores.

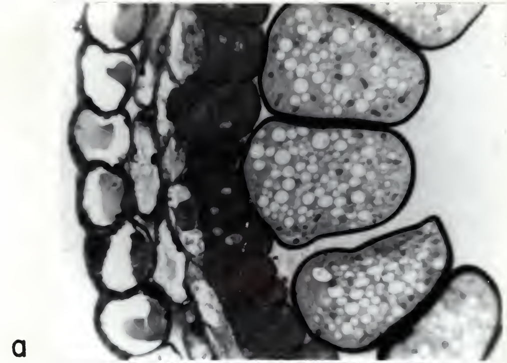
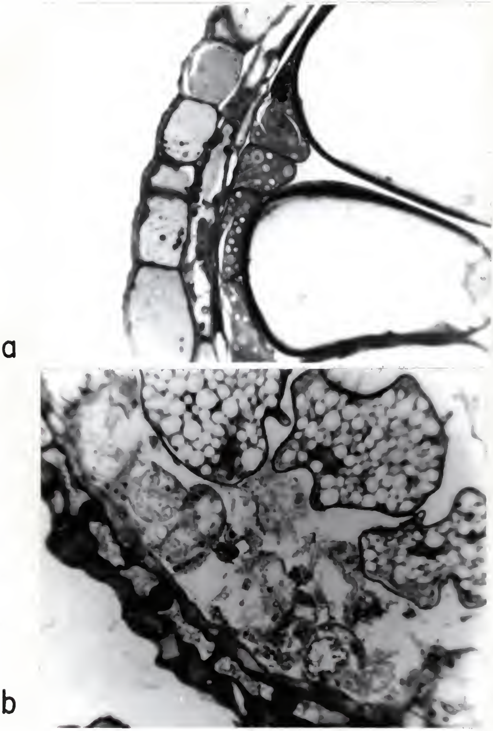

a
b

Figure 11. Sections of portions of fertile and sterile corn anthers at late 1-nucleate microspore stage at higher magnification. X 970. (a) is from fertile anther; (b) from sterile. The tapetum is disorganized in the sterile anther and intact, but compressed against anther wall, in the fertile.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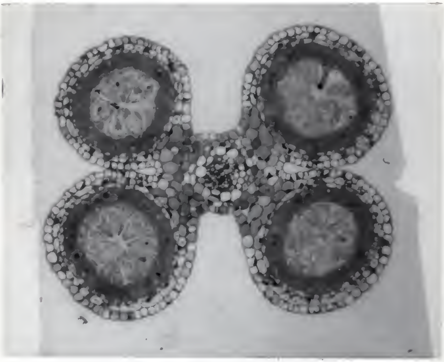


Figure 12. Cross section of entire corn anther showing arrangement of locules and central vascular bundle. X 190.

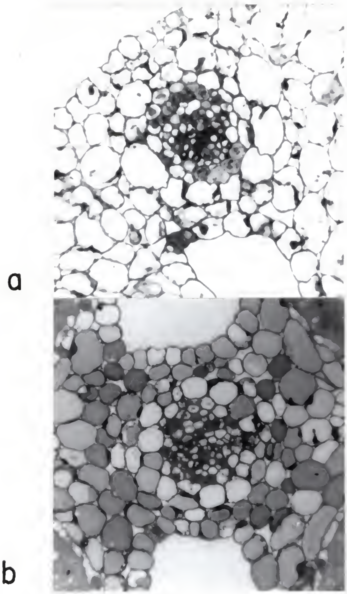


Figure 13. Sections of vascular bundles from fertile (a) and sterile (b) corn anthers at the tetrad stage. X 430. Xylem on the top and phloem at the bottom. No differences are observed at this time.

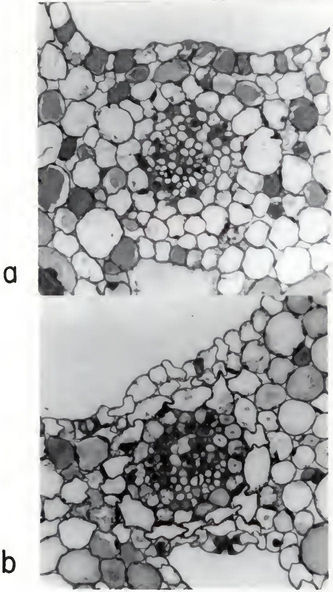


Figure 14. Sections of vascular bundles from fertile and sterile anthers at the intermediate microspore stage. X 430. The large sheath cells surrounding the vascular bundle are circular in outline in the fertile anther (a) but somewhat flattened in the sterile (b).

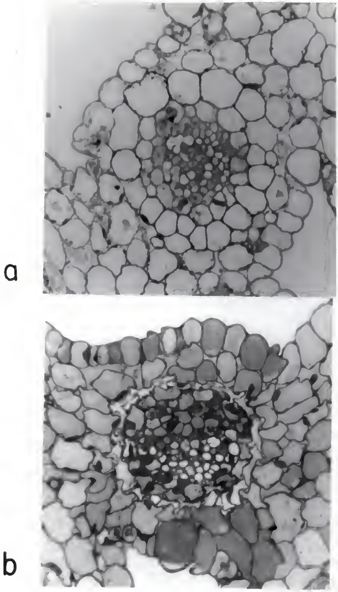


Figure 15. Sections of vascular bundles from fertile and sterile anthers at the late microspore stage. X 430. The surrounding layer of sheath cells has collapsed in the sterile anthers (b) but cells remain open in the fertile anther (a).

sterile corn anthers appear to differ in developmental patterns.

4.2.1 Differences in Organelle Development

Figures 16-21 are electron micrographs of portions of tapetal cells from fertile and sterile anthers at six progressively older stages of development. The top half of each plate (labeled a) shows tapetal cells of fertile anthers; the lower half (labeled b) shows tapetal cells at corresponding stages in sterile anthers. These stages correspond to those shown in Fig. 1 to 6 in Section 4.1. Fertile and sterile anthers in these series were given the same preparative treatments and were carefully paired, first by study of stained semithin sections under the light microscope, and finally, by electron microscope comparisons.

At the early stages of development (Fig. 16-17), tapetal mitochondria in both fertile and sterile anthers are large, with indistinct cristae and membranes. Fixation appears to be poor at this time, possibly because of slow fixative penetration into the solid locule core. Some signs of mitochondrial replication may be noted, and by the time of meiosis (Fig. 18) mitochondria become notably smaller. At this stage the outer membranes are sharp, the cristae are open and distinct, and the matrix is intermediate in density. At none of these stages, however, do mitochondria in fertile and sterile anthers differ in any distinct or consistent way.

At the tetrad stage, differences in mitochondrial structure become evident. The cristae in mitochondria of sterile tapeta (Fig. 19b) become disorganized, and the matrix is light and without structure. The bounding membranes remain distinct, however. In corresponding fertile tapeta (Fig. 19a), the cristae are distinct and the matrix darker. Tapetal mitochondria in fertile anthers at the young microspore stage (Fig. 20a) remain essentially unchanged, with sharp cristae and dark matrix. In the corresponding sterile anthers (Fig. 20b), mitochondria are disorganized internally and stain lightly.

At the intermediate microspore stage in sterile anthers, internal disorganization of mitochondria is advanced (Fig. 21b) with only traces of cristae remaining. The matrix is diffuse, and mitochondria have swollen to roughly twice their normal size. Mitochondria remain dense in corresponding fertile anthers (Fig. 21a) and have distinct cristae. Accurate comparisons of fertile and sterile anthers at later stages were difficult because of failure of the microspore nucleus to divide in sterile anthers and because of general tapetal and microspore breakdown.

The change in appearance of mitochondria in the tapeta of sterile anthers does not occur all at once; frequently sections at late meiosis or early tetrad stages are found which contain both normal and abnormal mitochondria. At the later microspore stages, however, as represented in Fig. 21b, all tapetal mitochondria in sterile anthers are abnormal.

Other tapetal organelles do not appear to differ in fertile and sterile anthers, at least not until a relatively late stage of anther breakdown. Proplastids in the tapetum of sterile anthers (Fig. 18b-21b) do not differ in any obvious way from those in fertile anthers (Fig. 18a-21a). Endoplasmic reticulum and ribosomes do not differ through the young microspore stage (Fig. 20a,b). At later stages, however, the tapetal cytoplasm and ribosomes largely disappear (Fig. 21b) and cause these anthers to differ conspicuously from corresponding fertile anthers (Fig. 21a).

The middle layer, which usually is persistent in corn through late anther development, follows the tapetum most closely in this regard. Between meiosis and the tetrad stage, mitochondria in this layer in sterile anthers increase in size, but cristae usually remain intact (Fig. 22b). After the tetrad stage, internal degeneration takes place, and mitochondria appear to be empty sacs bounded by double membranes (Fig. 23b). These structures may become so enlarged and degenerate in the middle layer as to make their identity uncertain (Fig. 24). In the middle layer of fertile anthers, mitochondria remain normal at corresponding stages (Fig. 22a,23a).

Organelles in the sporogenous tissue of fertile and sterile anthers do not differ significantly during the stages discussed above. Figures 25 and 26 show cytoplasm in sporogenous cells of fertile and sterile anthers at the time of meiosis and tetrad formation, respectively. Mitochondria and proplastids in fertile and sterile anthers are indistinguishable. Probably the same also is true at the young and intermediate microspore stages (Fig. 27 and 28); although there may be a tendency for more dark inclusions resembling osmiophilic bodies to appear in sterile proplastids and mitochondria at these stages (Fig. 28b). Mitochondria in the tapeta and middle layers of sterile anthers at these stages become degenerate, while those in the sporogenous tissue do not, or do so only to a lesser degree.

A notable increase in size of plastids in the endothecium occurs at the tetrad and young microspore stages and is correlated with starch deposition. This occurs and appears to be similar in both fertile and sterile anthers (Fig. 29).

In general, the similarities and differences in organelle behavior in anthers from fertile and sterile plants, as observed under the electron microscope, are borne out by measurements of size and number to be reported in Section 4.3.

4.2.2 Differences in Microspore Development

It was noted in Section 4.1.6 that young microspores in sterile anthers tend to have thinner walls than those in fertile anthers. This is seen at the electron microscope level to be the result of delayed and irregular sporopollenin deposition on microspores in sterile anthers.

Figures 27a,b show young fertile and sterile microspores shortly after the release from tetrads. Differences in exine formation are obvious. Figures 28a,b show corresponding exines at the intermediate microspore stage. The fertile microspore has an exine made up of a regular tectum and nexine and a well-developed system of channels (Fig. 28a). The exine of the sterile microspore is irregular and less developed and is without channels (Fig. 28b). The sterile exine may develop to the stage where some irregular channels form (Fig. 30b), but in general, it remains thin and irregular. Figure 30 shows about the maximum exine development observed in Tcms anthers. This compares to the normal exine in mature pollen in fertile anthers (Fig. 30a). The exines of sterile microspores always remain irregular and may fuse and cause microspore adhesion.

More tubular sacs and endoplasmic reticulum fragments also are observed under the plasma membranes of fertile than sterile microspores (Fig. 27a,b). Germ pores form in both fertile and sterile microspores. Those in steriles, however, are irregular and poorly developed. A thick intine is formed in fertile pollen (Fig. 30a), but has never been observed in sterile microspores. At time of flowering, the exine of sterile microspores is totally compressed (Fig. 31).

4.2.3 Discussion

The present study goes a step beyond those previously reported and critically invesigates the behavior of organelles (especially those bearing DNA) in male-sterile plants. These investigations, made with aid of the electron microscope and ultrathin sections, indicate that mitochondria at early stages of anther development are indistinguishable in fertile and sterile anthers.

By the tetrad and later stages, however, mitochondria in the tapetum and middle layer of sterile anthers become abnormal. They lose their cristae, become disorganized internally, and swell. Mitochondria in these tissues of fertile anthers,and subjected to the same preparative procedures, remain normal. This reaction is so pronounced that random ultrathin sections of anthers can be classified as fertile or sterile by this single criterion.

This abnormal mitochondrial behavior is recognizable early, before tapetal abnormalities are evident under the light microscope (Fig. 4a,b), and thus well before the general degenerative changes associated with male sterility become evident. This abnormal mitochondrial development has been observed in the tapetum and middle layers but not in the sporogenous cells or in other anther tissues. Other organelles, including plastids, endoplasmic reticulm, and ribosomes do not appear to differ in fertile and sterile anthers until after general degenerative changes begin in sterile anthers.

Earlier workers have tried to associate male sterility with cytoplasmic differences, beginning with the original work of Rhoades (1933). Using a Peruvian strain of corn (since lost), Rhoades noted differences between microspores from fertile and sterile plants in size, shape, and number of cytoplasmic elements, which he presumed to be plastids. Later, Rhoades (1950) postulated that mutated mitochondria are responsible for male sterility arising in connection with the iojap gene. This assumption was based largely on genetic evidence that plastids were not involved.

Other workers, also using the light microscope (Chang, 1954; Edwardson, 1955; Jones et al., 1957; Khoo and Stinson, 1957) have failed to observe cytological differences in fertile and male-sterile corn lines. At the present state of our knowledge, it probably is fair to say that cytological investigations based solely on the light microscope are not likely to yield critical evidence regarding finer cytoplasmic and organelle detail.

More recently, Edwardson (1962), using the electron microscope, observed differences in unidentified cytoplasmic inclusions in maintainer and Tcms corn roots and anthers. These inclusions at times were associated with membranes characteristic of mitochondria or plastids, but these structures were not specifically identified.

De Vries and Ie (1970), also using an electron microscope, observed no difference in cytoplasmic organelles in the tapetum of male-sterile and fertile wheat, but did observe differences in number and activity of of organelles in the cytoplasm of fertile and sterile microspores. Laser (1972b) reported that shape, size, and distribution of organelles were similar in fertile and sterile sorghum anthers until microspore abortion. Horner and Rogers (1974) found no disruption of organelles in the tapetum of cms *Capsicum* anthers.

All of these investigators have been plagued by fixation difficulties and by cause and effect relationships. The present study minimizes these problems by use of the best current light and electron microscope procedures and by making observations before general degenerative changes appear.

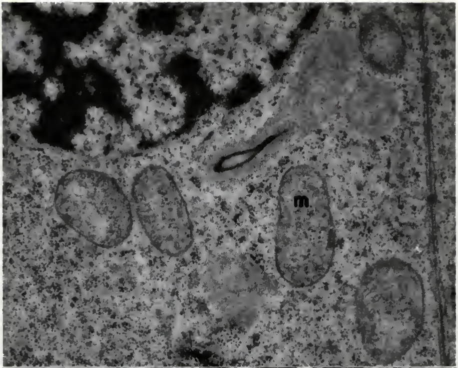
These observations increase our understanding of the mechanism of cytoplasmic male sterility by showing not only tapetal involvement, but that specific organelles in the tapetum may malfunction.

Differences in microspore architecture such as those observed in exine, intine, vacuolation, division or non-division of nucleus, starch deposition, and differences observed in anther behavior, including endothecial thickening, dehiscence, wall breakage between locules, anther exertion, and vascular differences, all occur at sequentially later stages of anther development than the mitochondrial degeneration discussed above and thus probably can be considered as secondary, or arrest effects, rather than causal.

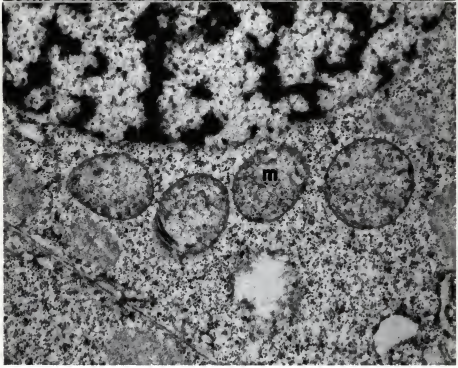
4.3 Changes in Organelle Size and Number During Growth of Fertile and Sterile Anthers

During the electron microscope studies of fertile and sterile anthers described in Section 4.2, differences in number and size of mitochondria were observed at the various growth stages and in the different tissues studied. Some apparent differences also were noted in plastid size. For this reason, and because of the possible relation to

Figure 16. Electron micrographs of mitochondria in tapetal cells from fertile (a) and sterile(b) corn anthers at the precallose stage (S_1). X 25,000. (m) indicates mitochondria.

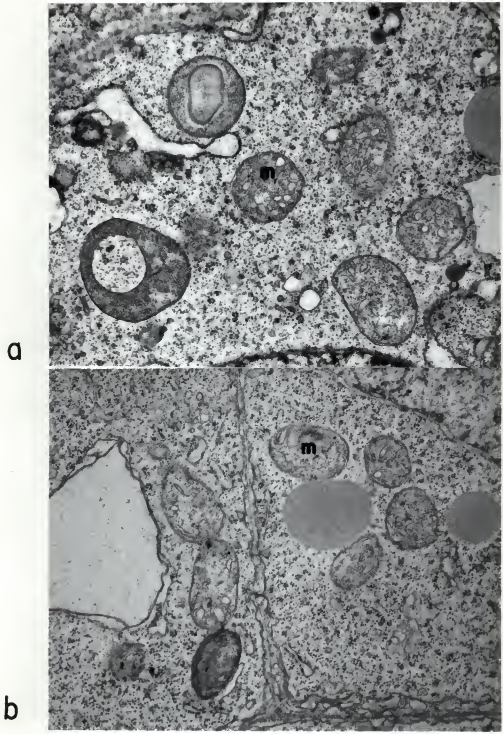


a



b

Figure 17. Micrographs of mitochondria in tapetal cells from fertile (a) and sterile (b) anthers at the central callose stage (S_2). X 25,000. (m) indicates mitochondria.


m
a
m
b

Figures 18. Micrographs of mitochondria and proplastids in tapetal cells from fertile (a) and sterile (b) anthers at meiosis (S_3). X25,000. (m) indicates mitochondria and (p) proplastids.

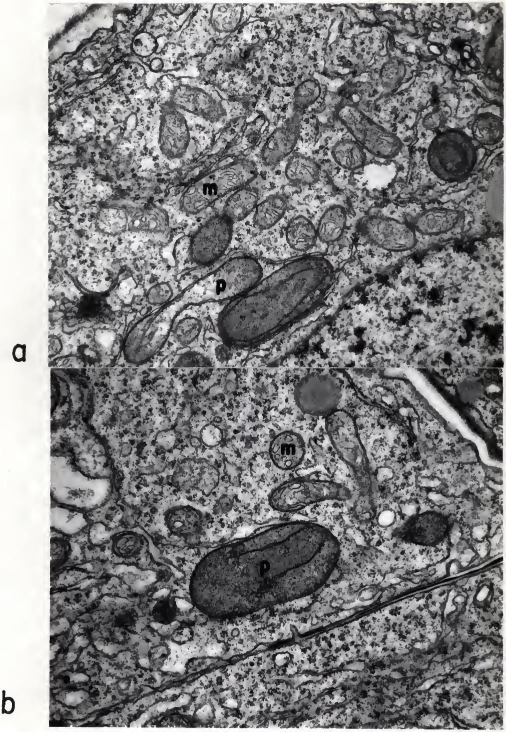
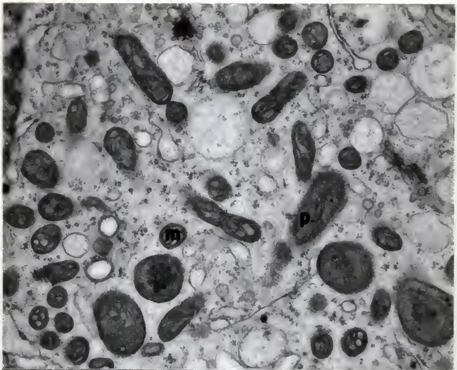
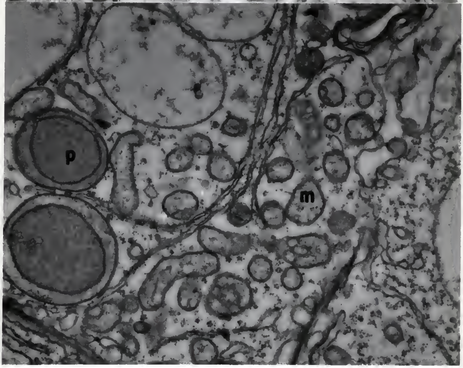

m
p
a
m
p
b

Figure 19. Mitochondria and plastids in tapetal cells from fertile (a) and sterile (b) anthers at the tetrad stage (S_4). X 25,000. Mitochondria in sterile anthers are losing internal structure and stain lightly. (m), mitochondria; (p), plastids.



a



b

Figure 20. Mitochondria and plastids in tapetal cells from fertile (a) and sterile (b) anthers shortly after release of microspores from the tetrads (S_5). X 25,000. (m), mitochondria; (p), plastids.

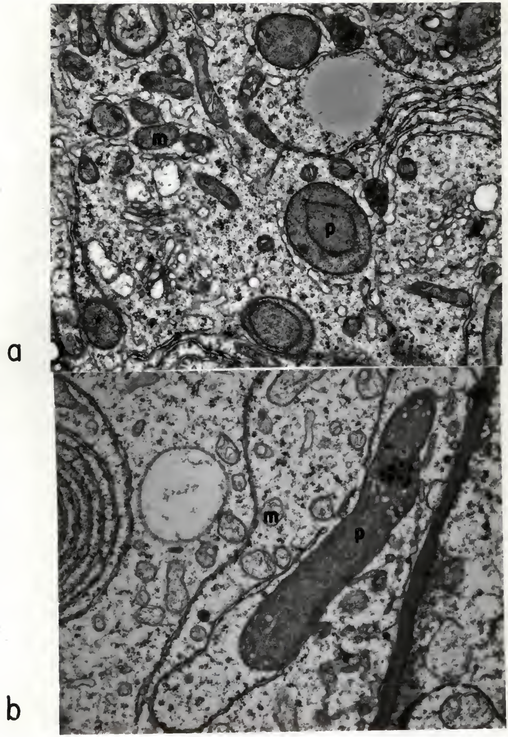

m
p
a
m
p
b

Figure 21. Micrographs of tapetal cells from fertile (a) and sterile (b) anthers at the intermediate microspore stage (S_6). X 25,000. Mitochondria in fertile anthers are normal; those in sterile anthers are enlarged and internal structure has largely disappeared. (m), mitochondria; (p), plastids.

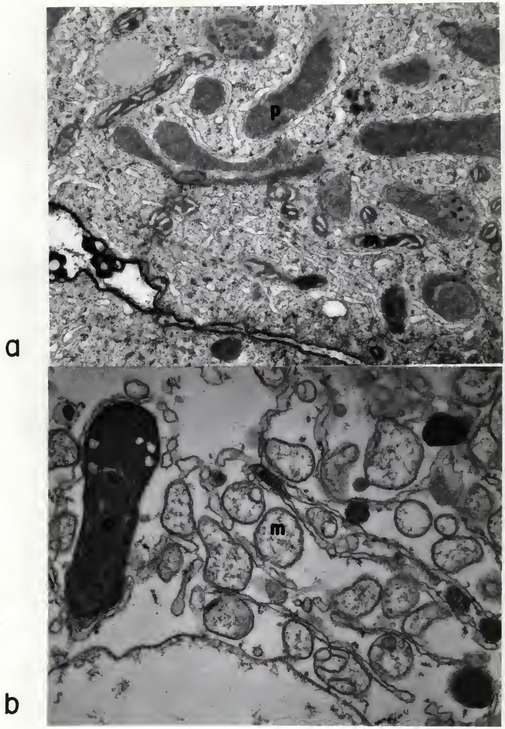

p
a
m
b

Figure 22. Electron micrographs of mitochondria and plastids in middle layer from fertile (a) and sterile (b) anthers at tetrad stage (S_4). X 25,000. Note that mitochondria (m) in sterile anthers are swollen and stain lightly.

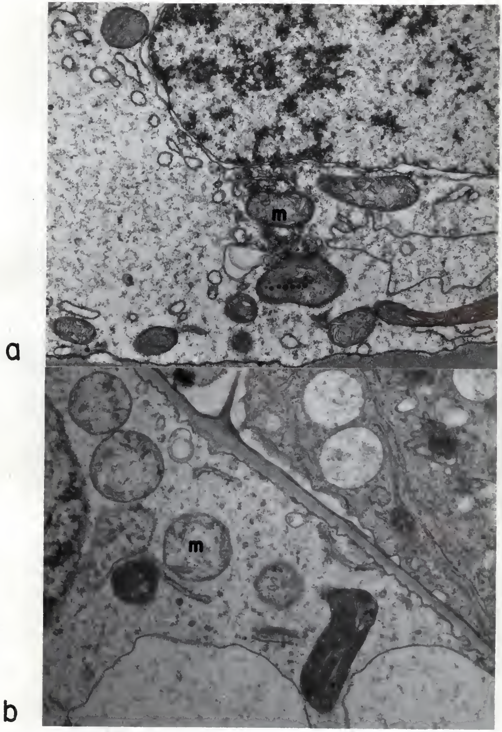
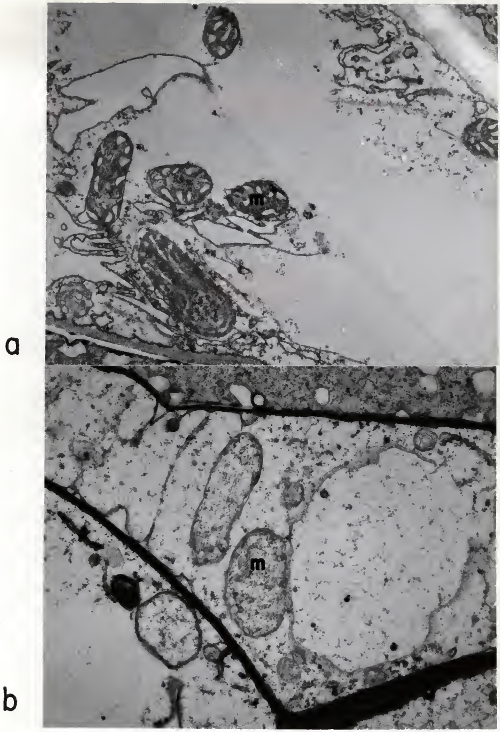

m
a
m
b

Figure 23. Micrographs of mitochondria in middle layer from fertile (a) and sterile (b) anthers at intermediate microspore stage (S_6). X 25,000. Mitochondria (m) in fertile anthers are normal; those in sterile anthers are swollen and have lost all internal structure.


m
a
m
b

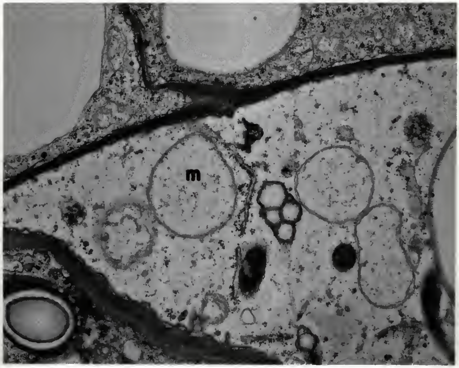
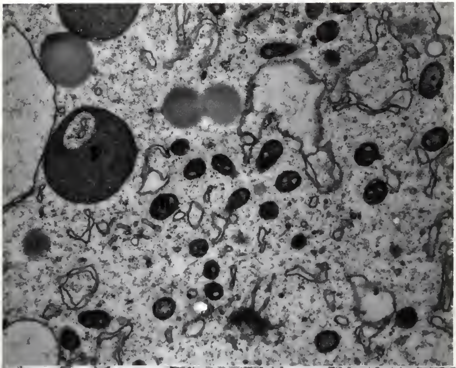
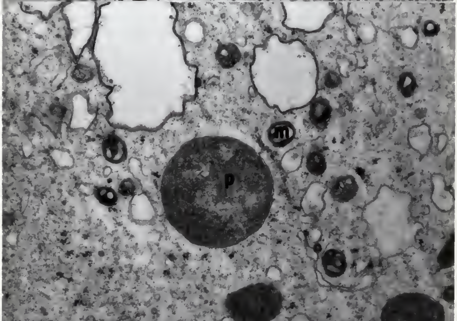


Figure 24. Micrograph of middle layer of sterile anther at young microspore stage (S_5). X 25,000. Mitochondria (m) have become swollen empty sacs, bounded by double membranes.

Figure 25. Micrographs of cytoplasm in sporogenous cells of fertile (a) and sterile (b) anthers at meiosis (S_3). X 25,000. (m), mitochondria; (p), plastids.



a



b

Figure 26. Mitochondria and plastids in sporogenous cells of fertile (a) and sterile (b) anthers at tetrad stage (S_4). X 25,000. (m), mitochondria; (p), plastids.

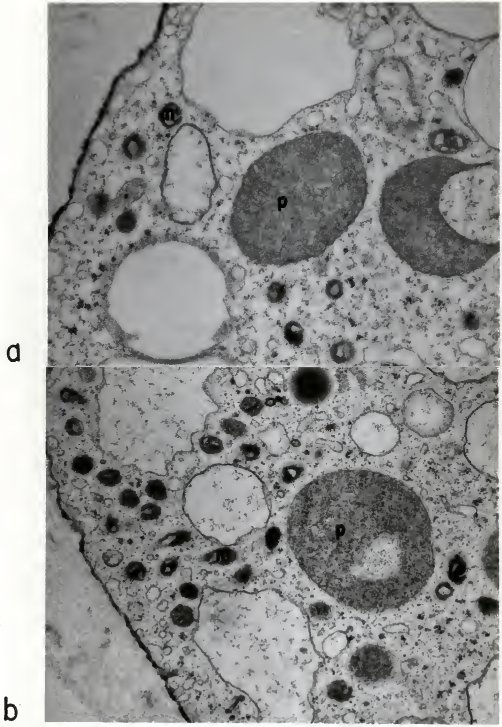

m
p
a
p
m
b

Figure 27. Mitochondria and plastids in microspores of fertile (a) and sterile (b) anthers shortly after release from tetrads (S_5). X 25,000. Note difference in exine (e) formation.

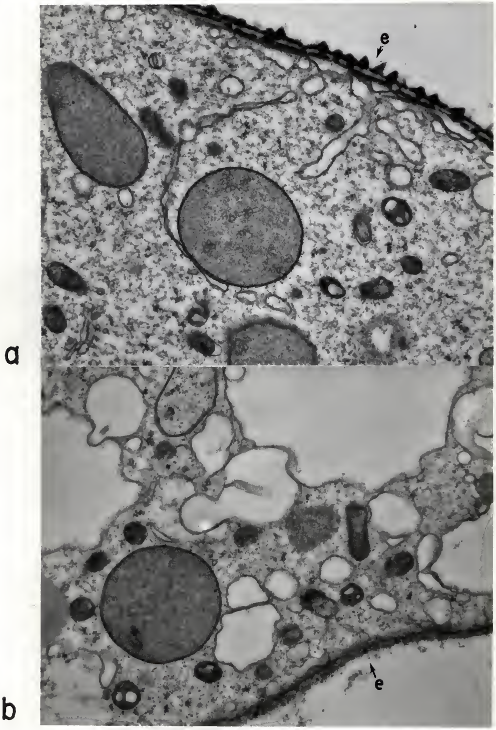

e
a
e
b

Figure 28. Mitochondria and plastids in sporogenous cells of fertile (a) and sterile (b) anthers at intermediate microspore stage (S_6). X25,000. Note differences in exine (e) formation and begining of mitochondrial breakdown in sterile anther.

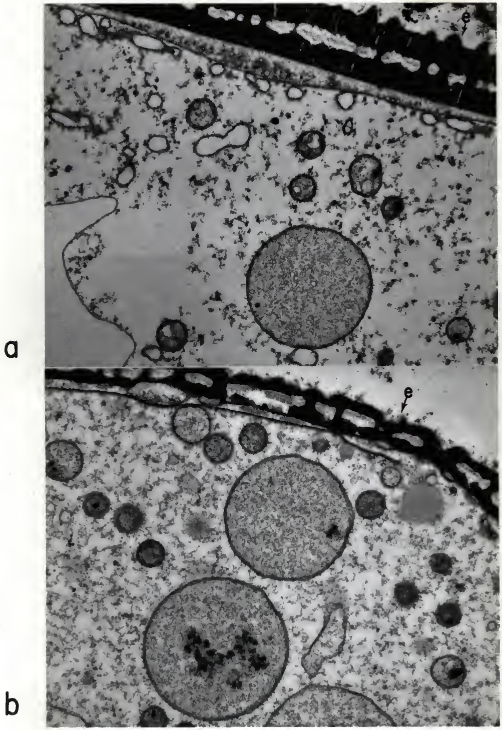

e
a
e
b

Figure 29. Electron micrographs of organelles in endothecial layer of fertile (a) and sterile (b) anthers at young microspore stage (S_5). X 25,000. Note enlargement of plastids and starch deposition.

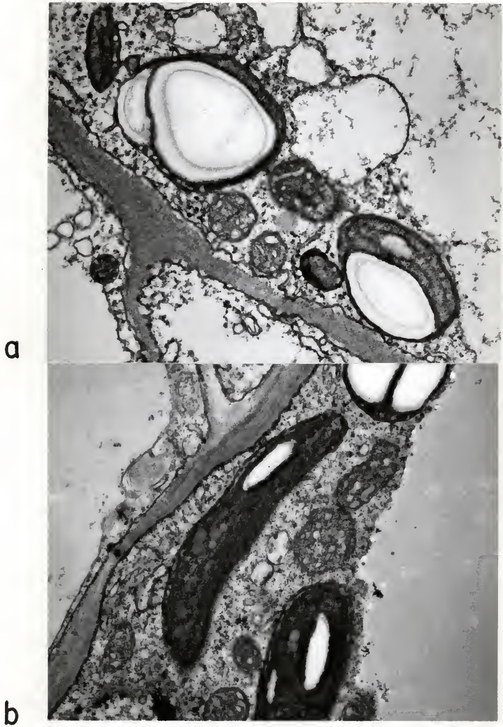
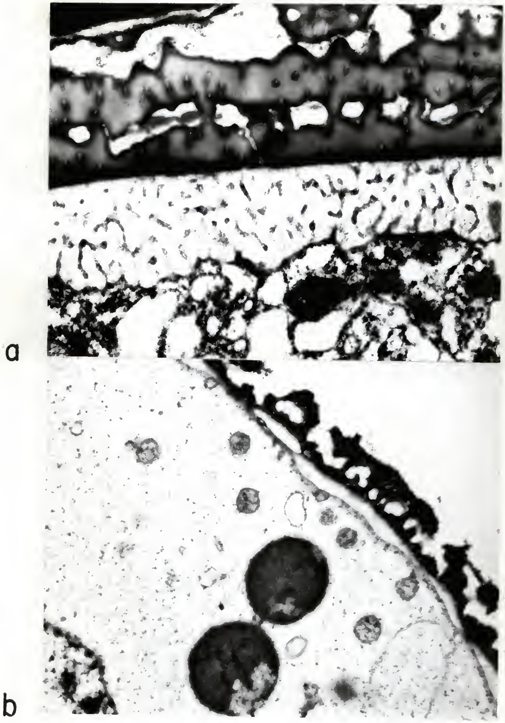

a
b

Figure 30. Detail of walls of fertile pollen (a) at maturity and sterile pollen (b) at late microspore stage. These are the conditions of walls at most advanced state of development in fertile and sterile anthers. X 25,000.


a
b

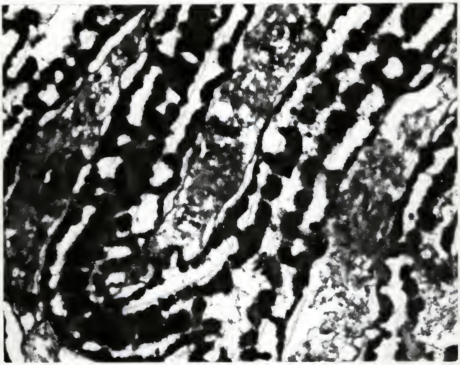


Figure 31. Exines of compressed microspores in sterile anthers at time of flowering. X 25,000.

male sterility, measurements and counts of these organelles were made as described under Section 3.

4.3.1 Mitochondrial Volume

Mitochondrial volume was studied in six tissues: sporogenous tissue (T_1), tapetum (T_2), middle layer (T_3), endothecium (T_4), epidermis (T_5), and vascular tissue (T_6) in fertile and sterile anthers for the six growth stages: precallose (S_1), central callose (S_2), meiosis (S_3), tetrad (S_4), young microspore (S_5), and intermediate microspore (S_6) (Table 1). An analysis of the data is given in Table 2.

The changes in mitochondrial volume with anther growth in all tissues are presented graphically in Fig. 32. There is a sharp decrease in size of mitochondria between precallose (S_1) and tetrad (S_4) and later stages for both sporogenous tissue and tapetum. These decreases are significant for both fertile and sterile anthers (Duncan's multiple range tests, Table 1). There is no similar decrease in mitochondrial size in any of the other tissues.

There is a significant increase in size of mitochondria in the sterile middle layer at stages S_4, S_5, and S_6 and a tendency for a similar increase in the tapetum from S_5 to S_6 (Fig. 32). These increases were noted in Section 4.2 and probably result from a swelling of mitochondria that accompanies mitochondrial breakdown in sterile anthers. No increases in size of mitochondria were noted in the tapetum or middle layers of corresponding fertile anthers.

Mitochondrial volume comparison among tissues at the same stage (Table 3) indicate those in the sporogenous tissue and tapetum at S_1 are significantly larger than those in other tissues in fertile anthers and larger than those in other tissues (except the endothecium) in sterile anthers. These differences disappear, however, by stages S_4 and S_6, and mitochondria in these cells actually become smaller than those in other tissues. In all these comparisons, sterile anthers differed significantly from fertile anthers in mitochondrial size only in the middle layer at the three later stages of development.

4.3.2 Mitochondrial Number per Unit Volume

The numbers of mitochondria per μm^3 for the various stages and tissues in fertile and sterile anthers are given in Table 4, and the analysis of variance of cytoplasms, tissues, and stages combined is shown in Table 2. Results of further analysis of each tissue are shown in Table 4.

Mitochondrial numbers for the six tissues are plotted in Fig. 33. A sharp increase in number of mitochondria was found in the tapetum and sporogenous tissue of both fertile and sterile anthers. This increase was statistically significant between S_1 and S_3 or S_5 for sporogenous tissue in both fertile and sterile anthers and between S_1 and S_4, S_5, and S_6 for tapetum in both fertile and sterile anthers. The apparent failure of mitochondria in the sporogenous tissue to increase in number during the later stages is due, at least in part, to the occurrence of meiosis at this time and the distribution of mitochondria among four cells. This increase in cell number is taken into consideration in calculations of mitochondrial number per cell (Section 4.3.3, below) and results in gradually increasing mitochondrial numbers in both tapetum and sporogenous tissue (Fig. 35).

There was no similar increase in number of mitochondria in the other tissues, except in the vascular tissue, where a slight increase also was noted.

The increase in number of mitochondria in tapetum and sporogenous tissue corresponds to the decrease in size noted in Fig. 32 and suggests that both changes are the results of rapid multiplication of mitochondria. The tendency for mitochondrial numbers in sterile tapeta to decrease sharply from S_5 to S_6 (Fig. 33) may be due to mitochondrial degeneration, noted above, to such an extent that they are no longer recognized as mitochondria under the electron microscope.

4.3.3 Mitochondrial Number per Cell

The numbers of mitochondria per cell in fertile and sterile anthers for the different tissues and growth stages are given in Table 5. Such estimates required calculations of cell volume (Table 6) plus a cell division factor (Table 7) and multiplying these by the number of mitochondria per μm^3 (Table 4). Because of the numerous measurements involved, cell volume calculations were limited to two tissues: tapetum and sporogenous cells (Table 6).

The data are presented graphically in Fig. 34 and Fig. 35. The sharp decrease in sporogenous cell volume between S_4 and S_5 in Fig. 34 is due to the separation of the four microspores from a single microspore mother cell as the result of the meiotic divisions. These divisions are compensated for by a cell division factor (Table 7) and are included in the curves shown in Fig. 35. These curves show a notable increase in number of mitochondria per cell between S_1 and S_6.

The calculations are given in Table 8 and indicate an average increase (mean of fertile and sterile anthers) of some 19-fold between S_1 and S_4 and 27-fold between S_1 and S_6 for sporogenous tissue. A similar increase of over 40-fold between S_1 and S_4 and almost 46-fold between S_1 and S_6 was found for the tapetum.

Although sporogenous cells are larger than tapetal cells and contain some 18 times more mitochondria at S_6 (Table 5), the rate of increase in number of mitochondria in the tapetum is greater (by a factor of approximately 2) than in the sporogenous tissue (Table 8, Tapetum/Sporogenous cells).

4.3.4 Plastid Volume

The same types of measurements and counts made for mitochondria also were made for proplastids in fertile and sterile anthers. Data for plastid volume are given in Table 9, and analysis of variance for cytoplasms, tissues, and stages combined is given in Table 2.

Significant differences in plastid volumes after analysis of variance and Duncan's multiple range test were found only in sporogenerous tissue and endothecium (Table 9). Plastid volumes for the six tissues are plotted in Fig. 36. The striking feature of these curves is the increase in volume of plastids at stages S_4 and S_5 in the endothecium. This increase is highly significant for both stage and tissue (Tables 9 and 10). Plastids in the other tissues remain relatively constant in size, except for a slight increase from S_2 to S_3 in sterile sporogenous tissue.

In no cases were significant differences in plastid volumes observed between fertile and sterile anthers.

4.3.5 Plastid Number per Unit Volume

Numbers of plastids per unit volume (μm^3) for the various tissues and stages in fertile and sterile anthers are given in Table 11. The data are shown graphically in Fig. 37, and an analysis of variance for cytoplasms, tissues, and stages combined is given in Table 2. Irregularities of the curves in Fig. 37 probably represent sampling error and result from the small numbers of plastids in some of the tissues. Both fertile-sterile and stage comparisons were found to be nonsignificant after analysis of variance (Table 11).

4.3.6 Plastid Number per Cell

Since no significant changes in plastid number per μm^3 were observed, calculations of plastid number per cell were not made. Such calculation would show little more than the increase in cell volume recorded in Fig. 34.

4.3.7 Discussion

No previous reports of organelle size and number as related to male-sterile cytoplasms have been encountered. Numerous studies have been carried out on organelles in normal plant tissues (Avers and King, 1960; Avers, 1962; Clowes and Juniper, 1964; Juniper and Clowes, 1965; Malone et al., 1974) and rather precise morphometric techniques have been developed (Baudhuin and Berthet, 1967;

Loud, 1968; Weibel, 1969; Larsen, 1970; and Mayhew, 1972).

With carefully paired ultrasections of fertile and sterile anthers at different growth stages already having been prepared for use in anther development comparisons, and with the facilities of the University Computing Center available, it seemed of value to make some organelle comparisons. These included calculations of mitochondrial and plastid size and number per unit volume and per cell for the six anther tissues at the six growth stages studied.

The most striking observation to come from these studies was a negative one: that no significant differences between organelles of fertile and sterile anthers that could be related in a causal way to pollen abortion were observed. A difference in mitochondrial size in fertile and sterile middle layer cells was noted at stages 4,5 and 6 (Fig. 32). This occurred late, however, and already has been discussed as resulting from mitochondrial swelling and breakdown in sterile anthers.

Another observation of note is the rapid division (as indicated by decrease in size and increase in numbers) that takes place in mitochondria of the tapetum and sporogenous cells during the early growth stages. Calculations indicate that numbers of mitochondria in the sporogenous cells increase almost 20-fold, and those in the tapetum some 40-fold, between precallose and tetrad stages. This behavior seems to be restricted to the tapetum and sporogenous tissue, because mitochondria in none of the other anther cells undergo a similar increase in numbers.

This period of rapid mitochondrial division coincides with the period of internal disorganization in sterile tapetal mitochondria noted in section 4.2, and it is tempting to speculate on a possible relationship: We know from the work of Levings and Pring (1976) on nucleic acid constitution, and from Miller and Koeppe (1971) and others on toxin reaction, that mitochondria from N and T plants are different. It also may be that they react differently to conditions in the anther. Perhaps N mitochondrial DNA is able to replicate or facilitate the synthesis of some essential product under the stress of rapid replication in the anther; a slightly altered DNA in T cytoplasm may not be able to do so. These would seem to agree with the general model of cytoplasmic male sterility proposed by Flavell (1974).

Measurements and counts of plastids showed no significant differences between fertile and sterile anthers in the tissues and stages studied. The only important difference observed in relation either to tissue or stage was a striking increase in plastid size during stages 3, 4, and 5 in the endothecium. This occurred to an equal degree in fertile and sterile anthers and appeared to be related to starch deposition.

TABLE 1. Calculated mean mitochondrial volumes (μm^3) and significance in six tissues of fertile and sterile corn anthers at six stages of development

Tissue		Developmental Stage Precallose (S1)	Central Callose (S2)	Meiosis (S3)	Tetrad (S4)	Young Microspore (S5)	Intermediate Microspore (S6)
Sporogenous Tissue[1] (T1)	F[3]	$.231^{a}$[4]	$.093^{b}$	$.009^{c}$	$.013^{c}$	$.011^{c}$	$.020^{c}$
	S[3]	$.172^{a}$	$.076^{b}$	$.011^{c}$	$.019^{c}$	$.019^{c}$	$.015^{c}$
Tapetum[1] (T2)	F	$.230^{a}$	$.184^{a}$	$.041^{b}$	$.014^{b}$	$.009^{b}$	$.008^{b}$
	S	$.225^{a}$	$.126^{b}$	$.044^{c}$	$.015^{c}$	$.013^{c}$	$.050^{c}$
Middle Layer[2] (T3)	F	$.071^{a}$	$.076^{a}$	$.063^{a}$	$.050^{a}$	$.068^{a}$	$.066^{a}$
	S	$.093^{a}$	$.055^{a}$	$.041^{a}$	$.209^{b}$	$.238^{b}$	$.278^{b}$
Endothecium[2] (T4)	F	.116	.100	.084	.083	.065	.082
	S	.114	.081	.050	.095	.059	.062
Epidermis[2] (T5)	F	.069	.116	.039	.069	.097	.057
	S	.068	.073	.042	.083	.062	.065
Vascular Tissue[2] (T6)	F	$.087^{ab}$	$.093^{a}$	$.043^{c}$	$.057^{bc}$	$.048^{c}$	$.035^{c}$
	S	$.077^{a}$	$.057^{ab}$	$.027^{b}$	$.045^{ab}$	$.036^{b}$	$.056^{ab}$

1) Values are mean mitochondrial volumes from three subsamples (different areas) from two replicates (different anthers).

2) Values are mean mitochondrial volumes from two subsamples (different areas) from two replicates (different anthers).

3) F and S indicate readings from sections of fertile and sterile anthers, respectively.

4) Means followed by the same letter within a tissue do not differ at the 5 percent level according to Duncan's multiple range test. Unmarked mean values are nonsignificant after F test in the analysis of variance.

TABLE 2. Mean squares and significance for organelle size and number per unit volume in fertile and sterile corn anthers (cytoplasms, tissues, and stages combined)

		Mitochondria		Plastids	
Source	DF	Volume	Number	Volume	Number
Cyto[1]	1	0.00187	0.19514	0.39533	0.01515
Stage	5	0.02351**	1.75733**	1.21935	0.00118
Cyto X Stage	5	0.00501	0.12974	0.11400	0.00119
Error A	12	0.00270	0.08034	0.38004	0.00326
Tissue	5	0.00945**	7.51821**	13.96365**	0.02695**
Cyto X Tissue	5	0.00920**	0.09801	0.00497	0.00303
Stage X Tissue	25	0.00919**	0.86693**	1.07395**	0.00284
Cyto X Stage X Tissue	25	0.00179**	0.12449*	0.09956	0.00146
Error B	60	0.00072	0.06344	0.21754	0.00187
Total	143	0.00411	0.54033	0.86612	0.00305

1) Cyto indicates type of cytoplasm (sterile or fertile).

*,** Significant at the 5 percent and 1 percent probability levels, respectively, as determined from an F test in the analysis of variance. Unmarked MS values are nonsignificant at these levels.

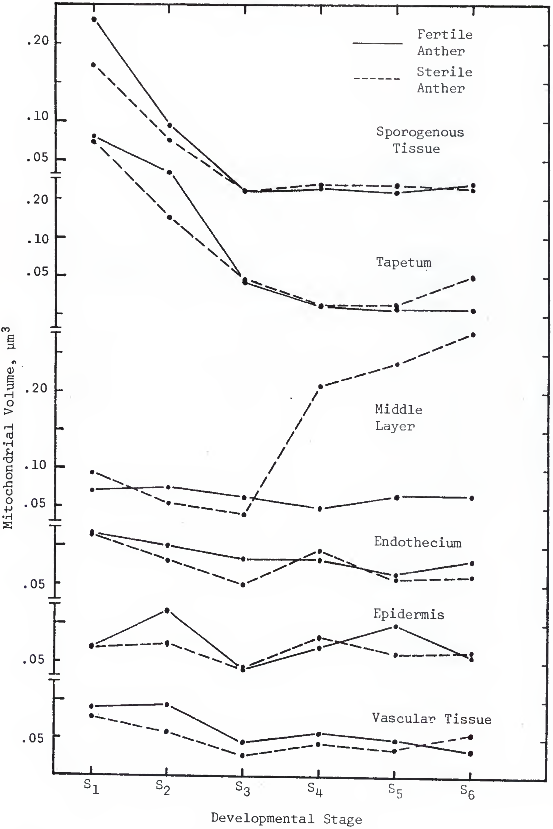


Figure 32. Relation of developmental stage and tissue to mitochondrial size in fertile and sterile corn anthers.

TABLE 3. Results of analysis of variance for mitochondrial volume and number per unit volume (cytoplasm and tissue in each stage)

	Stage	Source	DF	F value[1]	Duncan's Multiple Range test[2]	
Vol	S_1	Cyto[3]	1/43	0.87		
		Tissue	5/43	19.38**	F	$T_5\ T_3\ T_6\ T_4 \quad T_2\ T_1$
					S	$T_5\ T_6\ T_3\ T_4 \quad T_1\ T_2$
Vol	S_4	Cyto	1/11	13.61**		
		Tissue	5/11	26.53**	F	$T_1\ T_2\ T_3\ T_6\ T_5\ T_4$
					S	$T_2\ T_1\ T_6\ T_5\ T_4\ T_3$
Vol	S_6	Cyto	1/11	11.55**		
		Tissue	5/11	14.09**	F	All nonsignificant
					S	$T_1\ T_2\ T_6\ T_4\ T_5\ T_3$
NV	S_1	Cyto	1/43	0.28		
		Tissue	5/43	12.81**	F	$T_2\ T_4\ T_6\ T_3 \quad T_5\ T_1$
					S	$T_4\ T_3\ T_2\ T_6 \quad T_5\ T_1$
NV	S_4	Cyto	1/11	1.64		
		Tissue	5/11	19.28**	F	$T_4\ T_5\ T_3\ T_6 \quad T_1\ T_2$
					S	$T_3\ T_4\ T_5\ T_6 \quad T_1\ T_2$
NV	S_6	Cyto	1/43	8.49**		
		Tissue	5/43	37.69**	F	$T_4\ T_3\ T_5\ T_6\ T_1\ T_2$
					S	$T_4\ T_5\ T_3\ T_6 \quad T_1\ T_2$

---

[1] *, ** Significant at the 5 percent and 1 percent probability levels, respectively. Unmarked F values are nonsignificant at these levels.

[2] Tissues joined by the same line are not significantly different at the 5 percent probability level according to Duncan's multiple range test.

[3] Cyto indicates type of cytoplasm (sterile or fertile).

TABLE 4. Mitochondrial number per unit volume (μm^3) and significance in six tissues of fertile and sterile corn anthers at six developmental stages

		Developmental Stage					
Tissue		Precallose (S1)	Central Callose (S2)	Meiosis (S3)	Tetrad (S4)	Young Microspore (S5)	Intermediate Microspore (S6)
Sporogenous Tissue[1] (T1)	F[3]	0.552^{a}[4]	0.592^{a}	2.282^{bc}	2.111^{bc}	2.400^{c}	1.343^{ab}
	S[3]	0.536^{a}	1.182^{ab}	1.781^{b}	1.387^{ab}	1.724^{b}	1.476^{ab}
Tapetum[1] (T2)	F	0.234^{a}	0.260^{a}	0.889^{a}	2.241^{b}	2.622^{b}	3.036^{b}
	S	0.276^{a}	0.373^{a}	0.915^{ab}	2.116^{c}	2.477^{c}	1.575^{bc}
Middle Layer[2] (T3)	F	0.296	0.354	0.271	0.381	0.306	0.331
	S	0.265	0.298	0.272	0.155	0.611	0.418
Endothecium[2] (T4)	F	0.253	0.223	0.131	0.211	0.239	0.266
	S	0.189	0.309	0.309	0.230	0.263	0.253
Epidermis[2] (T5)	F	0.507	0.456	0.469	0.356	0.181	0.378
	S	0.406	0.348	0.385	0.250	0.264	0.276
Vascular Tissue[2] (T6)	F	0.269^{a}	0.418^{a}	0.415^{a}	0.386^{a}	0.817^{b}	0.785^{b}
	S	0.310^{a}	0.588^{ab}	0.881^{b}	0.451^{a}	0.596^{ab}	0.467^{a}

1) Values are mean mitochondrial number per unit volume from three subsamples (different areas) from two replicates (different anthers).
2) Values are mean mitochondrial number per unit volume from two subsamples (different areas) from two replicates (different anthers).
3) F and S indicate readings from sections of fertile and sterile anthers, respectively.
4) Means followed by the same letter within a tissue do not differ at the 5 percent level according to Duncan's multiple range test. Unmarked mean values are nonsignificant after F test in the analysis of variance.

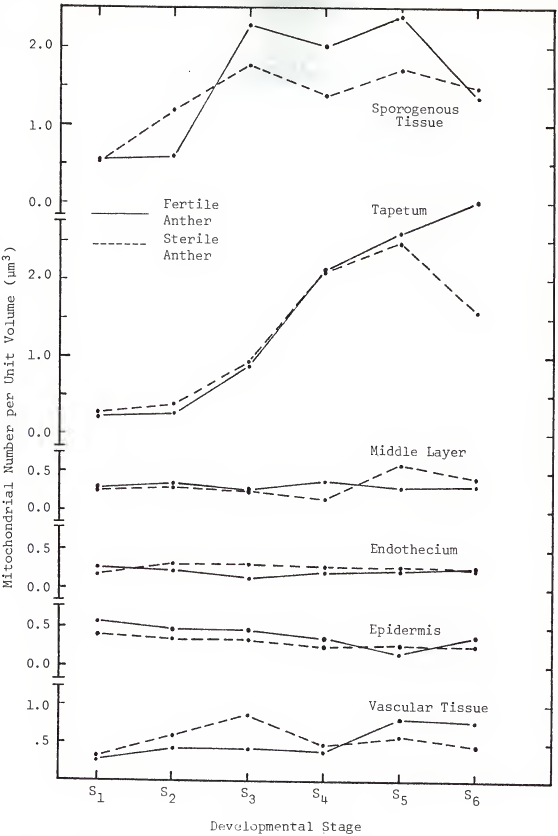


Figure 33. Relation of stage and tissue to mitochondrial number per unit volume in fertile and sterile corn anthers.

TABLE 5. Calculated number of mitochondria per cell for tapetal and sporogenous tissue of fertile and sterile anthers for six developmental stages

		Developmental Stage					
Tissue		Precallose (S1)	Central Callose (S2)	Meiosis (S3)	Tetrad (S4)	Young Microspore (S5)	Intermediate Microspore (S6)
Sporogenous Tissue (T1)	F	6,794[1]	11,484	38,956	156,699	208,300	188,133
	Mean	6,680	20,228	51,497	126,401	165,718	178,135
	S	6,566	28,973	64,038	96,104	123,135	168,137
Tapetum (T2)	F	191	184	2,052	9,267	8,378	13,006
	Mean	216	304	2,362	8,814	9,793	9,916
	S	240	424	2,672	8,360	11,207	6,826

1) Values represent products of mitochondrial number per unit volume (Table 4) by calculated cell volume (Table 6) by cell division factor (Table 7).

TABLE 6. Calculated cell volumes (μm^3) in sporogenous tissue and tapetum of fertile and sterile anthers at six developmental stages

Tissue		Developmental Stage Precallose (S1)	Central Callose (S2)	Meiosis (S3)	Tetrad (S4)	Young Microspore (S5)	Intermediate Microspore (S6)
Sporogenous Tissue (T1)	F	12,301[1]	19,415	30,408	74,230	21,701	35,012
	S	12,246	24,505	35,970	69,330	17,858	28,482
Tapetum (T2)	F	819	693	2,098	3,335	2,497	3,483
	S	872	881	1,826	2,863	3,481	3,468

1) Values represent means of products of cell cross-sectional areas (from three subsamples, two replicates) by cell length (from two subsamples, two replicates).

TABLE 7. Cell division factor for sporogenous tissue and tapetum of fertile and sterile anthers at six developmental stages

		Developmental Stage					
Tissue		Precallose (S1)	Central Callose (S2)	Meiosis (S3)	Tetrad (S4)	Young Microspore (S5)	Intermediate Microspore (S6)
Sporogenous Tissue							
Cell Numbers	F	1	1	1	1	4	4
	S	1	1	1	1	4	4
Cell Division Factor	F	1	1	1	1	4.0	4.0
	S	1	1	1	1	4.0	4.0
Tapetum							
Cell Numbers	F	32.5	33.0	35.7	40.3	41.7	39.8
	S	28.8	38.0	46.0	39.8	37.3	36.0
	Mean	30.7	35.5	40.8	40.1	39.5	37.9
Cell Division[1] Factor	F	1	1.0	1.1	1.2	1.3	1.2
	S	1	1.3	1.6	1.4	1.3	1.3
	Mean	1	1.2	1.4	1.3	1.3	1.2

1) Values represent ratios of cell number at each stage (mean of three subsamples and two replicates) compared to cell number at precallose (S_1).

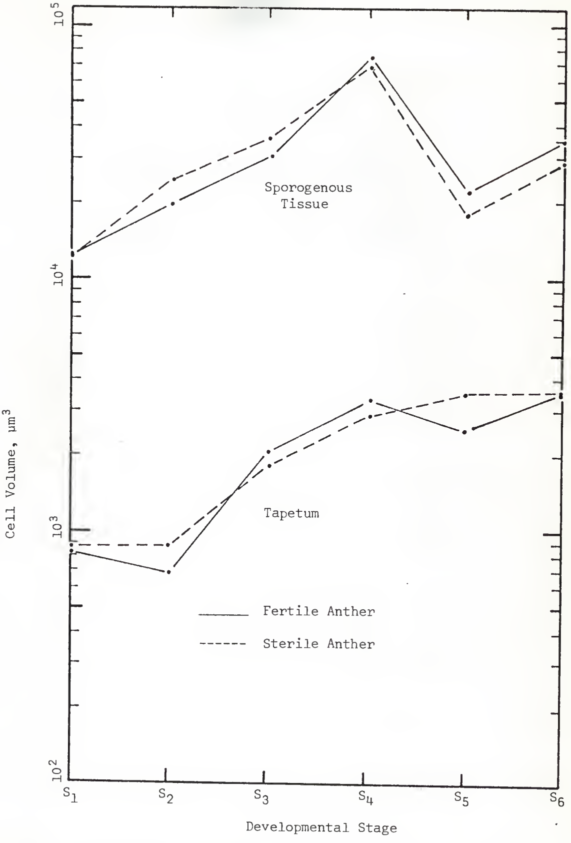


Figure 34. Calculated cell volumes in sporogenous tissue and tapetum at six developmental stages in fertile and sterile corn anthers. Plotted on semi-log scale.

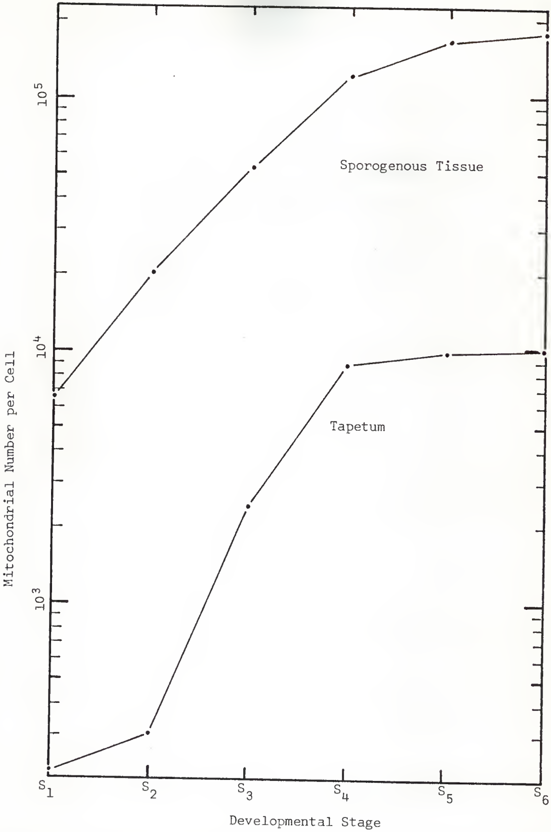


Figure 35. Calculated numbers of mitochondria per cell for sporogenous tissue and tapetum in corn anthers. Mean of values for fertile and sterile anthers plotted on semi-log scale.

TABLE 8. Comparison of mean mitochondrial number per cell (combined fertile and sterile) for sporogenous tissue and tapetum at stages S_1, S_4, and S_6

Tissue	Developmental Stage Precallose (S1)	Tetrad (S4)	Intermediate microspore (S6)	$\frac{(S4)}{(S1)}$	$\frac{(S6)}{(S1)}$
Sporogenous Tissue	6,680	126,401	178,135	18.9	26.7
Tapetum	216	8,814	9,916	40.8	45.9
			$\frac{\text{Tapetum}}{\text{Sporogenous Tissue}}$	2.2	1.7

TABLE 9. Calculated mean plastid volumes (μm^3) and significance in six tissues of fertile and sterile corn anthers at six developmental stages

Tissue		Developmental Stage Precallose (S1)	Central Callose (S2)	Meiosis (S3)	Tetrad (S4)	Young Microspore (S5)	Intermediate Microspore (S6)
Sporogenous Tissue[1] (T1)	F[3]	.686^{a}[4]	.675^{a}	1.007^{a}	1.103^{a}	1.100^{a}	.895^{a}
	S	.583^{ab}	.410^{b}	1.144^{a}	.844^{ab}	.796^{ab}	1.118^{ab}
Tapetum[1] (T2)	F	.398	.686	.221	.437	.335	.443
	S	.324	.215	.180	.277	.257	.729
Middle Layer[2] (T3)	F	.299	.454	.147	.158	.104	.378
	S	.367	.209	.076	.263	.304	.311
Endothecium[2] (T4)	F	.661^{a}	.861^{a}	2.791^{ab}	4.150^{b}	3.272^{b}	2.132^{ab}
	S	.925^{a}	1.168^{a}	2.398^{ab}	3.144^{ab}	3.871^{b}	1.724^{ab}
Epidermis[2] (T5)	F	.469	.472	.534	.560	.845	.400
	S	.375	.143	.144	.707	.928	.371
Vascular Tissue[2] (T6)	F	.456	.483	.391	.230	.232	.440
	S	.540	.269	.122	.071	.128	.080

1) Values are mean plastid volumes from three subsamples (different areas) from two replicates (different anthers).

2) Values are mean plastid volumes from two subsamples (different areas) from two replicates (different anthers).

3) F and S indicate readings from sections of fertile and sterile anthers, respectively.

4) Means followed by the same letter within a tissue do not differ at the 5 percent level according to Duncan's multiple range test. Unmarked mean values are nonsignificant after F test of analysis of variance.

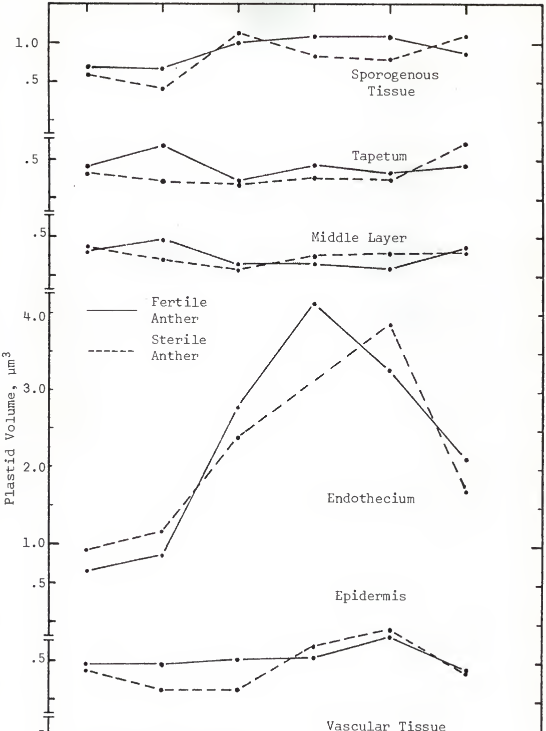


Figure 36. Relation of developmental stage and tissue to plastid size in fertile and sterile corn anthers.

TABLE 10. Results of analysis of variance for plastid volume (cytoplasm and tissue in each stage)

Stage	Source	DF	F value[1]	Duncan's multiple range test[2]	
S_3	Cyto[3]	1/11	0.60		
	Tissue	5/11	16.93**	F	$\underline{T_3\ T_2\ T_6\ T_5\ T_1}\ T_4$
				S	$\underline{T_3\ T_6\ T_5\ T_2\ T_1}\ T_4$
S_4	Cyto	1/11	0.73		
	Tissue	5/11	15.70**	F	$\underline{T_3\ T_6\ T_2\ T_5\ T_1}\ T_4$
S_5	Cyto	1/43	0.00	S	$\underline{T_6\ T_3\ T_2\ T_5\ T_1}\ T_4$
	Tissue	5/43	19.66**	F	$\underline{T_3\ T_6\ T_2\ T_5\ T_1}\ T_4$
				S	$\underline{T_6\ T_2\ T_3\ T_1\ T_5}\ T_4$
S_6	Cyto	1/11	0.01		
	Tissue	5/11	5.28*	F	$\underline{T_3\ T_5\ T_6\ T_2\ T_1}\ T_4$
				S	$\underline{T_6\ T_3\ T_5\ \underline{T_2\ T_1}}\underline{\ T_4}$

1 *, ** Significant at the 5 percent and 1 percent probability levels, respectively. Unmarked F values are non-significant at these levels.

2 Tissues joined by the same line are not significantly different at the 5 percent probability level according to Duncan's multiple range test.

3 Cyto indicates type of cytoplasm (sterile or fertile).

TABLE 11. Plastid number per unit volume (μm^3) and significance in six tissues of fertile and sterile anthers at six developmental stages

Tissue		Developmental Stage					
		Precallose (S1)	Central Callose (S2)	Meiosis (S3)	Tetrad (S4)	Young Microspore (S5)	Intermediate Microspore (S6)
Sporogenous Tissue[1] (T1)	F[3]	0.097[4]	0.080	0.058	0.046	0.044	0.070
	S[3]	0.084	0.129	0.045	0.073	0.084	0.047
Tapetum[1] (T2)	F	0.119	0.078	0.177	0.121	0.159	0.107
	S	0.119	0.147	0.275	0.187	0.168	0.117
Middle Layer[2] (T3)	F	0.092	0.074	0.066	0.073	0.140	0.077
	S	0.119	0.083	0.112	0.082	0.103	0.061
Endothecium[2] (T4)	F	0.070	0.066	0.061	0.041	0.052	0.062
	S	0.079	0.055	0.041	0.062	0.048	0.046
Epidermis[2] (T5)	F	0.082	0.106	0.068	0.085	0.054	0.107
	S	0.101	0.153	0.108	0.053	0.080	0.084
Vascular Tissue[2] (T6)	F	0.071	0.076	0.130	0.071	0.154	0.073
	S	0.109	0.129	0.138	0.192	0.132	0.201

1) Values are mean plastid number per unit volume from three subsamples (different areas) from two replicates (different anthers).

2) Values are mean plastid number per unit volume from two subsamples (different areas) from two replicates (different anthers).

3) F and S indicate readings from sections of fertile and sterile anthers, respectively.

4) Means followed by the same letter within a tissue do not differ at the 5 percent level according to Duncan's multiple range test. Unmarked mean values are nonsignificant after F test of analysis of variance.

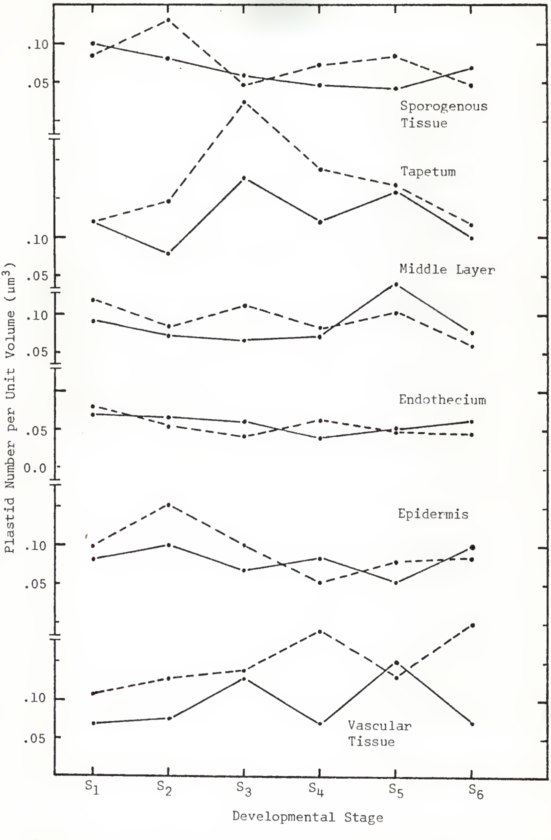


Figure 37. Relation of stage and tissue to plastid number per unit volume in fertile and sterile corn anthers.

4.4 Miscellaneous Investigations

In addition to the investigations reported above, other, but related studies were made of mitochondrial behavior in the following materials: 1) F_6N and T anthers, 2) restored F_{44}T anthers, and 3) F_{44}T leaf and ovule tissues. These were carried out at the electron microscope level and employed the same procedures as the studies reported above.

4.4.1 Mitochondrial Behavior in F_6N and F_6T Anthers

F_6 is another Florida inbred that is available with both normal and T cytoplasms. Since F_6T has the same sterile Texas cytoplasm as F_{44}T, but a different nuclear genome, it seemed of value to make a limited study to determine if mitochondrial behavior is similar to, or different from, that in F_{44}T anthers.

Figure 38 shows portions of the tapetum from F_6N(a) and F_6T(b) anthers at the intermediate microspore stage. Mitochondria in F_6T anthers show the same loss of cristae and internal disorganization that was observed in F_{44}T anthers. Mitochondria in the tapetum from F_6N anthers do not show this abnormality (Fig. 38a). No organelle measurements or counts were made from these anthers, but mitochondria appear very similar in size and shape to those in F_{44} tissues.

4.4.2 Mitochondrial Behavior in Restored $F_{44}T$ Anthers

When male-sterile $F_{44}T$ plants are crossed as females with homozygous restorer lines (those with Rf_1 and Rf_2 nuclear genes), the offspring are all fertile. These fertile offspring carry the same sterile cytoplasm as the female parent, but have been made fertile by the action of nuclear genes contribured by the pollen parent.

Anthers from the F_1 hybrids were studied, therefore, to determine if mitochondrial degeneration in the tapetum is a characteristic of the T cytoplasm and is still found in the fertile hybrids, or if this is corrected by restorer genes in the hybrid.

Figures 39a,b, and c show portions of the tapetum of anthers from $F_{44}N$, restored $F_{44}T$ plants, and $F_{44}T$ sterile plants at the tetrad stage. Mitochondria in the $F_{44}T$ restored anthers appear normal and are indistinguishable from those found in $F_{44}N$ plants.

4.4.3 Mitochondrial Behavior in Leaves and Ovules of $F_{44}T$ Plants

Since abnormal behavior of mitochondria was observed in certain cells of T cytoplasmic male-sterile anthers, it seemed of interest to examine other tissues for mitochondrial abnormalities. This especially is true since differences in mitochondrial structure in nucellar cells of fertile and male-sterile corn lines have been reported

(Palilova et al., 1967; Turbin et al., 1968).

Figures 40a and b show mitochondria in fertile and T sterile leaf tissues, and Figures 41a and b show portions of nucellar cells from fertile and male-sterile plants.

No differences in mitochondrial behavior in fertile and sterile plants were observed in either leaf or nucellar tissues. Also, there was no indication of the internal mitochondrial disorganization found in the tapetum of male-sterile anthers, or of the size and structural differences reported by the Russian workers.

4.4.4 Discussion

Lines F_6T and $F_{44}T$ both produce sterile offspring when crossed with their respective maintainers; both lines also produce fertile offspring when crossed with restorers. It was decided, therefore, to study mitochondrial behavior in these plants and crosses to determine if the breakdown observed in $F_{44}T$ lines also occurs in F_6T, and to investigate what happens in plants with T cytoplasm and restorer genes. This should indicate if mitochondrial breakdown always accompanies sterility, or if it is a characteristic of the T cytoplasm or of a particular nuclear constitution or combination.

Mitochondria were found to degenerate in unrestored T cytoplasm whether with F_{44} or F_6 nuclei. This reaction thus is not unique to the F_{44} genome. Mitochondria in the tapetum of plants with T cytoplasm and nuclei containing restorer genes are normal. Mitochondrial breakdown thus is not inherent in the T cytoplasm.

This behavior parallels the observed fertile-sterile reaction in such crosses and indicates that mitochondrial degeneration is directly related to sterility and is controlled by specific cytoplasm - nuclear gene interaction.

Since fertile and male-sterile plants are not distinguishable before flowering and have very similar growth rates, and since mitochondria are known to play a fundamental role in plant metabolism, it would seem unlikely that mitochondria in Tcms plants would be structurally or functionally altered in somatic tissues. No such differences have been observed in the present study, but have been reported by Palilova et al. (1967) and Turbin et al. (1968). The latter findings remain unexplained, except possibly on the basis of techniques employed.

Finally, it should be noted that mitochondrial degeneration, similar to that found in the present study, also has been observed in interspecific Gossypium hybrids (Phillips and Reid, 1975). The first evidence of abnormality in Gossypium was development of electron-transparent areas in the matrix and a reduction in number of cristae. This led to progressive degeneration of all internal mitochondrial structure. Other organelles appeared to be normal. Although the abnormalities in Gossypium occur in the cotyledons of seedlings and leaves of young hybrid plants and result in tumor formation and necrosis, the pattern of mitochondrial breakdown is strikingly similar to that in corn anthers. Since cytoplasmic male sterility is thought to have appeared after wide crosses in many plant species (Edwardson, 1970), the similarity of mitochondrial breakdown in corn anthers and in cotton hybrids may support the notion of a hybrid origin for Tcms corn.

Figure 38. Micrographs of cytoplasm of tapetal cells from F6N (a) and F6T (b) anthers at intermediate microspore stage (S6). X25,000. Note degeneration of mitochondria in the F6T sterile anther.

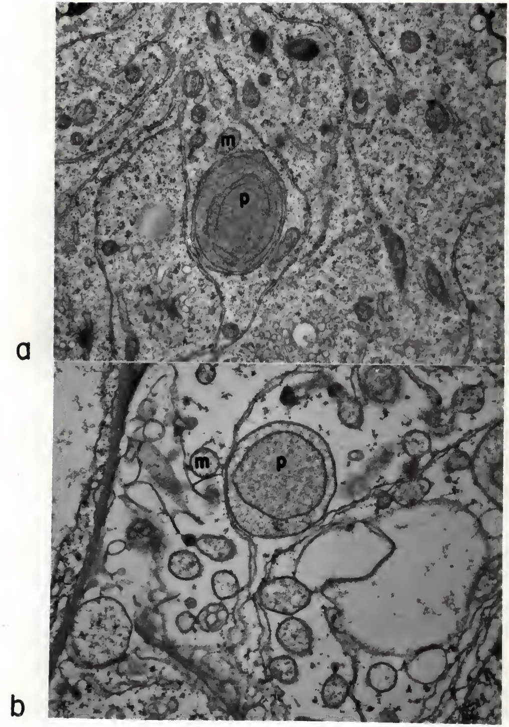

m
p
a
m
p
b

Figure 39. Mitochondria in tapetal cells of N (a), T-restored (b), and T-sterile (c) anthers of line F44 at tetrad stage (S4). X 25,000.

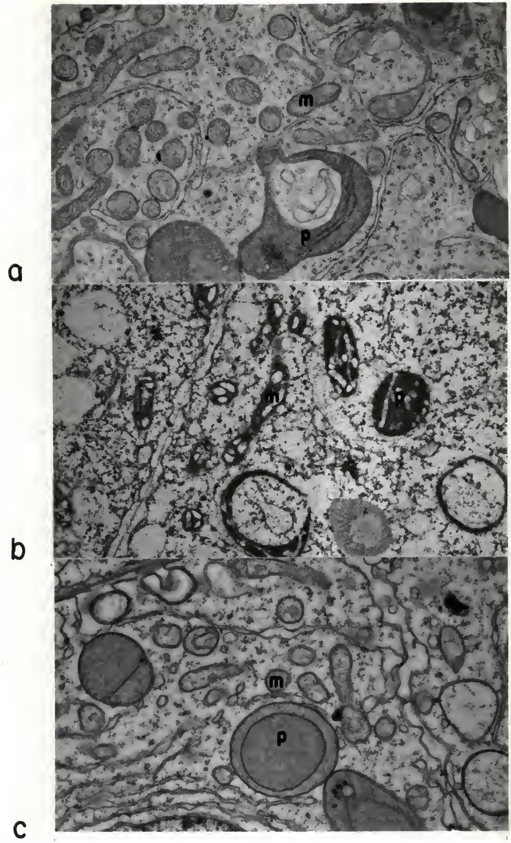

m
p
a
m
p
b
m
p
c

Figure 40. Micrographs of mitochondria and plastids in leaf bundle sheath cells from fertile (a) and sterile (b) F44 plants. X 25,000.

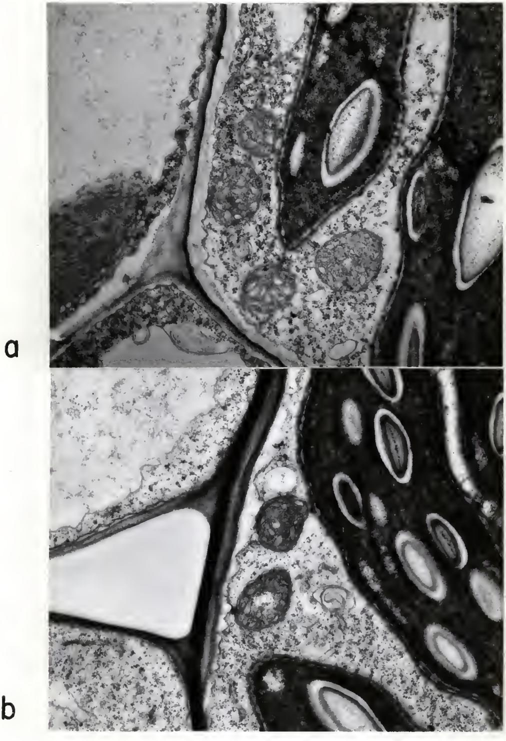
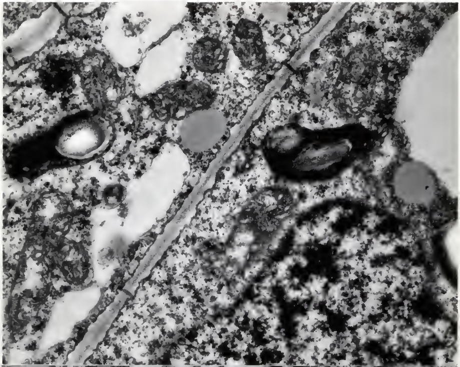
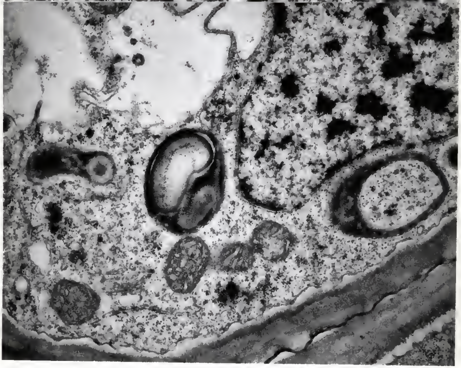

a
b

Figure 41. Micrographs of mitochondria in nucellar cells from fertile (a) and sterile (b) plants of line F44. X 25,000.



a



b

APPENDICES

APPENDIX I. Relation of coefficient of configuration (β) and ratio of axes of particles (λ). Data cited from Weibel (1969).

λ	β
1.0	1.38
1.2	1.39
1.4	1.43
1.6	1.47
1.8	1.53
2.0	1.58
2.2	1.65
2.4	1.73
2.6	1.79
2.8	1.87
3.0	1.94
3.4	2.10
4.0	2.36
5.0	2.80

APPENDIX II. Computer program for calculating organelle volume and number per unit volume (N_V).

$$\text{Vol} = 4/3\,\pi a b^2; \quad N_V = \frac{K\,{N_A}^{3/2}}{\beta {V_V}^{1/2}}$$

```
$JOB
    INTEGER BLOCK(8),C(6,5)
    DIMENSION NCA(6),M(6),A(50),B(50),V(50),X(14),BT(14)
    READ(5,120) (X(I),I=1,14)
    READ(5,120) (BT(I),I=1,14)
    DO 5 I=1,6
5   READ(5,100) (C(I,J),J=1,5)
    DO 65 II=1,7
    READ(5,100) (BLOCK(I),I=1,8)
    READ(5,110) (NCA(I),I=1,6)
    WRITE(6,200) (BLOCK(I),I=1,8)
    WRITE(6,220)
    DO 55 NC=1,6
    KN=NCA(NC)
    READ (5,110) (M(I),I=1,KN)
    WRITE(6,210) (C(NC,J),J=1,5)
    AVMN=0.0
    ADVA=0.0
    AANV=0.0
    AT=0.0
    DO 45 K=1,KN
    N=M(K)
    READ(5,120) (A(I),I=1,N),(B(I),I=1,N)
    VMN=0.0
    DO 15 I=1,N
    V(I)=A(I)*B(I)*B(I)*0.52359833
15   VMN=VMN+V(I)/N
    DVA=0.0
    DO 20 I=1,N
20   DVA=DVA+ABS(VMN-V(I))/N
    D=(VMN*1.90986093)**0.33333333
    SA=0.0
    SB=0.0
    VV=0.0
    DO 16 I=1,N
    SA=SA+A(I)
    SB=SB+B(I)
    VV=0.03141*A(I)*B(I)+VV
16  CONTINUE
```

```
     R=SA/SB
     DO 25 I=1,14
     IF(R-X(I)) 25,25,31
31   L=I
     GO TO 32
25   CONTINUE
32   BETA=BT(L)+(R-X(L))*(BT(L+1)-BT(L))/(X(L+1)-X(L))
     ANV=1.05*(0.04*N)**1.5/BETA/VV**0.5
     WRITE(6,230) K,N,VMN,DVA,ANV,T,R,BETA
     T=ANV*VMN
     IF(KN-1) 45,45,40
40   AVMN=AVMN+VMN/KN
     ADVA=ADVA+DVA/KN
     AANV=AANV+ANV/KN
     AT=AT+T/KN
45   CONTINUE
     IF(KN-1) 55,55,35
35   WRITE(6,240) AVMN,ADVA,AANV,AT
55   CONTINUE
65   CONTINUE
100  FORMAT(8A4)
110  FORMAT(6I2)
120  FORMAT(24F3.2)
200  FORMAT('1',38X,8A4,///)
210  FORMAT(/,20X,5A4)
220  FORMAT(34X,'NUMBER  MEAN VOL.  MEAN DVA.    NV
        VOL. FRAC.  LAMDA     l   BETA')
230  FORMAT(23X,'RECORD #',I1,4X,I2,5X,F6.3,6X,F6.3,3X,
       F5.3,4X,F6.3,12(4X,F4.2))
240  FORMAT(/,23X,'AVERAGE VALUE',7X,F6.3,F6.3,3X,F5.3,
       4X,F6.3)
     STOP
     END
$ENTRY
```

APPENDIX III. SAS programs for analysis of variance.

1) For cytoplasm[1] and stage in each tissue

```
PROC ANOVA;
CLASSES REP VAR STAGE SUBSAM;
MODEL VOL NV PL_VOL PL_NV = REP VAR/STAGE;
POOL 'ERROR A' REP*VAR REP*STAGE REP*VAR*STAGE;
TEST VAR/STAGE BY 'ERROR A';
```

2.) For cytoplasm and tissue at each stage

```
PROC ANOVA;
CLASSES REP VAR TISUE SUBSAM;
MODEL VOL NV PL_VOL PL_NV = REP VAR/TISUE;
POOL 'ERROR A' REP*VAR REP*TISUE REP*VAR*TISUE;
TEST VAR/TISUE BY 'ERROR A';
```

3) For cytoplasms tissues, and stages combined

```
PROC ANOVA;
CLASSES REP VAR STAGE TISSUE;
MODEL VOL NV PL_VOL PL_NV = REP VAR/STAGE;
POOL 'ERROR A' REP*VAR/STAGE;
MODEL = TISSUE TISSUE*VAR /STAGE:
POOL 'ERROR B' REP*TISSUE REP*TISSUE*VAR/STAGE;
TEST VAR/STAGE BY 'ERROR A';
TEST TISSUE TISSUE*VAR/STAGE BY 'ERROR B';
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[1] VAR = cytoplasm

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BIOGRAPHICAL SKETCH

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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